CLINICAL STUDY

Intramyocellular triglyceride content in man, influence of sex, obesity and glycaemic control

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Abstract

Objective: It remains unknown whether sex impacts on intramyocellular triglyceride (IMTG) in obesity, as has been shown in non-obese subjects, and, if so, whether this may have implications on the association between IMTG and insulin sensitivity.

Subject and methods: A muscle biopsy from vastus lateralis was obtained in 27 obese women (body mass index (BMI) = 35.5 ± 0.8 kg/m²; mean ± S.E.M., percentage of body fat (PBF) = 44 ± 1, n = 7 impaired fasting glucose, n = 7 type 2 diabetes), 20 obese men (BMI = 35.8 ± 0.8 kg/m²; PBF = 33 ± 1, n = 4 impaired-fasting-glucose; n = 6 type 2 diabetes) and 12 lean sedentary healthy individuals (controls; n = 7 women, BMI = 21.8 ± 0.7 kg/m²; PBF = 20 ± 2; n = 5 men, BMI = 23.6 ± 0.5 kg/m²; PBF = 13 ± 2). IMTG was determined by chromatography.

Results: IMTG was increased twofold in obese women compared to obese men, lean men and lean women respectively (21.9 ± 2.4 mg/g wet weight, 10.9 ± 2.4 mg/g, P < 0.001). Among obese subjects of either gender IMTG did not increase along with reduced glycaemic control in terms of impaired fasting glucose and diabetes. Plasma insulin levels, which were similar among obese women with different glycaemic control levels, but much lower in lean women, paralleled the changes in IMTG among women. PBF was associated with IMTG in all subjects (P < 0.001). In a linear model, sex (P < 0.05) and PBF (P < 0.05) independently explained variation in IMTG. Plasma free fatty acids (FFA) correlated with IMTG in all subjects (P < 0.005).

Conclusion: Obese women display twice as much IMTG as obese men matched for BMI. Increased IMTG could be a pathophysiologial element or a mere physiological phenomenon in feminine obesity ensuing prior to impaired glycaemic control, but associated with increased body fat, circulating FFA and insulin.

Introduction

Neutral fat in muscle plays an important role as oxidative substrate during and following physical activity (1). Athletes and well-trained individuals possess a high concentration of intramyocellular triglyceride (IMTG) (2), which after a training bout may be reduced by 20–30% during the recovery period where muscle glycogen depots build up (3). In trained individuals, IMTG is preferentially allocated in close vicinity to the mitochondria. This is in contrast to the more dispersed distribution of IMTG in sedentary individuals (4). In sedentary individuals, IMTG turnover is slow (5). In sedentary individuals, it has been shown that increased IMTG is associated with insulin resistance (6–9). This could be due to increased availability of fatty acids in the muscle, and thus higher availability of fatty acids for β-oxidation (10). Recently, it has been suggested that by-products of intramyocellular lipids as diacylglycerol and ceramides play a detrimental role for insulin signalling pathways pivotal for insulin action, which would help to explain the association of IMTG and insulin resistance (11).

An association between obesity and IMTG may be a result of, increased free fatty acids (FFA) availability, increased influx of FFA in muscle, low lipid oxidation capacity, and facilitated triglyceride assimilation (12). De novo lipogenesis from increased glucose availability in the muscle may be another potential mechanism of increased IMTG (13). Increased lipoprotein lipase activity in obesity may facilitate intramyocellular FFA content and, secondary to this, IMTG content. A two- to threefold greater lipoprotein lipase gene expression in skeletal muscle has been demonstrated in sedentary women compared to sedentary men and may indicate an increased potential for increasing muscular...
lipoprotein activity in females (14). A gender difference in IMTG may also be caused, at least in part, by sex related differences in hormone sensitive lipase activity in the muscle (15).

Sedentary lean women exhibit twice as much IMTG as matched men (4, 16). To the best of our knowledge, no study has addressed whether these gender differences can also be found in obese subjects. Demonstration of a gender effect on IMTG in obesity would potentially impact our understanding of the molecular mechanisms behind obesity and insulin resistance. Accordingly, the present study was undertaken to investigate IMTG concentration in a group of obese men and women, who displayed a broad range of insulin resistance and glycaemic control.

**Subjects and methods**

**Subjects**

To investigate whether gender may impact on IMTG content in obesity we merged data from subjects, who have participated in two previous studies in which skeletal muscle biopsies were obtained (17, 18). This was done to achieve adequate statistical power. However, analysis on IMTG content was performed exclusively for the present study. Fifteen non-diabetic obese subjects were recruited from one of the studies (18), and 44 subjects, including lean control subjects from the other study (17). In total 59 subjects were available for study: 47 obese subjects (n=27 women) and 12 lean control subjects (n=7 women). Criteria for inclusion of subjects have been outlined in detail (17, 18). In brief, medication for dyslipidemia, including supplementation with fish oil, lipid lowering medication and insulin therapy for diabetes mellitus (DM) was not allowed. Diabetes was defined as two independent measurements of fasting plasma glucose ≥ 7.0 mmol/l or present use of anti-diabetic medication. Among the 13 patients with diabetes on inclusion only two were on oral antidiabetic medication. Both patients paused this medication 7 days prior to examination and harvesting of muscle biopsies. Only one subject was on anti-hypertensive medication. All included subjects were sedentary. All subjects reported stable weight before the study. All obese subjects had a systolic blood pressure at 155 mmHg or lower and a diastolic blood pressure at 95 mmHg or lower. Obese subjects of body mass index (BMI) levels from 28 to 45 kg/m² were included. All obese subjects had a systolic blood pressure at 155 mmHg or lower and a diastolic blood pressure at 95 mmHg or lower. All included subjects were white Danes. Informed written consent was obtained in accordance with Helsinki Declaration II. The local ethical committee approved the study.

**Anthropometric measurements**

Body weight and height were measured on a calibrated scale. Waist circumference was measured in the standing position between the top of the iliac crest and the lower rib margin on each side, while the patient exhaled and with the tape parallel to the floor. Hip circumference was measured in the horizontal plane at the level of the maximal extension of the buttocks. Measurements of weight, height, waist and hip were carried out in duplicate and mean values were noted. Waist circumference was used as an indicator of abdominal fat distribution (19). Total fat mass was determined by the tetrapolar bioelectric impedance technique using the Holtain body composition analyser (Holtain Ltd, Crosswell, UK); four electrodes (AccuSensor CarboCone M55, Lynn Medical, Bloomfield Hills, MI, USA) were placed carefully to ensure reproducible measurements (20). Impedance, weight, height, gender and age were used to calculate the amount of body fat using validated equations (21).

**Blood sampling and assays**

Subjects were told to not perform strenuous physical exercise 48 h before blood sampling. After an overnight fast (10 h) and 15 min of rest, a catheter was inserted in the antecubital vein for blood sampling. Handling and measurement of blood samples have been previously described (17, 18). Samples were analysed for glucose, insulin, C-peptide, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein cholesterol, triglyceride, FFA, and HbA1c by use of standardized procedures. FFA was measures in a subpopulation of 44 subjects only.

**Muscle biopsy and measurement of IMTG**

A percutaneous muscle biopsy was obtained under local anaesthesia using a Bergström needle (Depuy, Phoenix, AZ, USA) from the vastus lateralis. The specimen was immediately and carefully dissected free of visible connective tissue, fat and blood and frozen in liquid nitrogen and stored at −80 °C until assayed. Extraction of muscle triglycerides in general followed the principle described by Folch et al. (22) and the actual procedure has been described in detail (18). In brief, samples of skeletal muscle tissue were added to the internal standard of C15:0 phosphatidylcholine followed by extraction of the total lipid material with chloroform/methanol, 2:1, vol/vol, during homogenization with an Ultra Turrax homogenizer. The extracted lipids were separated into phospholipids and triglycerides by thin-layer chromatography using a pre-manufactured silica plate (Merck). The silica gel bands containing the triglycerides were scraped off the thin-layer chromatography plate and extracted from the silica gel. The fatty acid profile was determined by gas–liquid
The calculation was based on the S.D. of 6 mg IMTG/g calculated as weight/height$^2$ (kg/m$^2$).

**Calculations**

The homeostasis model assessment insulin resistance index (HOMA-IR) derives an estimate of whole body insulin sensitivity from fasting glucose and insulin concentrations: HOMA-IR = fasting insulin (μU/ml) × fasting plasma glucose (mM)/22.5 (23). BMI was calculated as weight/height$^2$ (kg/m$^2$).

**Statistics**

All data are expressed as mean ± S.E.M. if not otherwise indicated. To compare the distribution between independent groups, a one-way ANOVA test was applied. Pearson correlation coefficients were obtained to quantify associations between variables. A multiple linear regression model was applied to evaluate potential independence of significant predictors of IMTG concentration. Normality of distributions was tested for before applying these tests. Given a sample size less than $n=7$, a low number, which hampers validation of normality of a distribution, a non-parametric test, e.g. the Kruskal–Wallis test, was applied. The sample size of obese women and obese men was chosen to achieve 80% power to detect a gender difference of 25% at significance level $P<0.05$. The calculation was based on the S.D. of 6 mg IMTG/g wet muscle tissue, which was obtained previously by us in 12 obese women and five obese men (18). Because gender difference in IMTG among sedentary lean subjects has been found to be as high as 50%, the detection level of gender difference in IMTG of 25%, according to the power calculation above, may be relevant (4). All statistical analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Significance was accepted for $P<0.05$. For 0.05 $<P<0.10$ a trend was indicated. $P>0.10$ was indicated as not significant (ns).

**Results**

It was observed that obese women displayed a twofold greater IMTG content compared to obese men, lean women and lean men, respectively (Fig. 1). Table 1 gives anthropometric and biochemical comparison between groups. Notably, obese women demonstrated a gynoid phenotype by a much lower waist hip ratio than obese men, who displayed an android fat distribution. Obese women matched carefully for BMI and age to obese men displayed a body fat percentage that was 11% greater than these men.

When obese women were categorized according to glycaemic control, i.e. normal fasting glucose (NFG) versus impaired fasting glucose (IFG) and DM, it appeared that IMTG content did not differ between these glycaemic indices (Table 2). Also when data from obese women with IFG and DM were merged to obtain a larger sample of subjects with glucose metabolic aberration ($n=14$) and thereafter compared to obese women with NFG ($n=13$) still no difference appeared in IMTG level between these sub-groups (data not shown). However, compared to lean women with NFG ($n=7$), obese women with NFG ($n=13$) or with IFG/DM ($n=14$) had a twofold greater IMTG content ($P=0.032$ and $P=0.015$), respectively. Plasma insulin levels, which were similar among obese women with different glycaemic indices, but much lower in lean women, thus paralleled the changes in IMTG in women (Table 2). However, insulin in women did not correlate significantly with IMTG ($r=0.25$, $P=ns$, $n=34$), and neither did HOMA-IR ($r=0.22$, $P=ns$, $n=34$).

By contrast to women, no differences in IMTG content was observed between lean versus obese men (Table 3). In addition, there were no differences in IMTG between obese men according to categories of glycaemic control. This also holds true when the subgroups of obese men with IFG and DM were merged to obtain a larger sample of obese men with glucose metabolic aberration ($n=10$) to compare with the obese men with NFG (data not shown). Plasma insulin did not parallel levels of IMTG
content in men. Neither insulin nor HOMA-IR correlated significantly with IMTG levels in men (r = 0.26, P = ns and r = 0.19, P = ns, n = 25).

Notably, percentage of body fat (PBF) correlated significantly with IMTG content in obese subjects (r = 0.54, P < 0.001, Fig. 2). In obese women, this correlation was also significant (r = 0.39, P < 0.05, n = 27) but not in obese men (r = 0.18, P = ns, n = 20).

By including all subjects (n = 59) in the analysis, the correlation between IMTG and PBF was r = 0.46, P < 0.001. In all women IMTG and PBF correlated significantly (r = 0.51, P = 0.002, n = 34), but not in all men (r = 0.04, P = ns, n = 25). In all women BMI and IMTG correlated significantly (r = 0.48, P = 0.004, n = 34), but not in obese women only (r = 0.31, P = ns, n = 27). There were no significant correlations between BMI and IMTG in men. The waist-to-hip ratio (WHR) did not show correlation with IMTG in the group of obese women (r = 0.04, P = ns, n = 27) nor in obese men (r = 0.25, P = ns, n = 20), but when the groups of obese men and women were merged a significant inverse correlation between WHR and IMTG was shown (r = −0.42, P < 0.005, n = 47), which probably signifies that a gynoid fat distribution of obese women may confer increased IMTG in perpendicular muscle tissue compared to obese men with an android fat distribution.

In a subset of 44 subjects (n = 17 obese women, n = 7 lean women, n = 15 obese men and n = 5 lean men) from the study in which FFA measurements were

### Table 1 Intramyocellular triglyceride, anthropometry and biochemistry of obese women compared with obese men. Data for lean women and men are also given.

<table>
<thead>
<tr>
<th></th>
<th>Obese women</th>
<th>Obese men</th>
<th>P obese women versus obese men</th>
<th>Lean women</th>
<th>Lean men</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (s.e.m.)</td>
<td>Mean (s.e.m.)</td>
<td></td>
<td>Mean (s.e.m.)</td>
<td>Mean (s.e.m.)</td>
</tr>
<tr>
<td>Total number/number of T2DM</td>
<td>27/7</td>
<td>20/6</td>
<td>0.0003</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>IMTG (mg/g wet weight)</td>
<td>21.9 (2.4)</td>
<td>10.9 (1.5)</td>
<td>0.0003</td>
<td>10.9 (2.7)*</td>
<td>9.8 (2.1)*</td>
</tr>
<tr>
<td>Size of muscle biopsy analyzed (mg)</td>
<td>77 (7)</td>
<td>64 (4)</td>
<td>ns</td>
<td>74 (6)</td>
<td>62 (4)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48 (2)</td>
<td>49 (2)</td>
<td>ns</td>
<td>48 (3)</td>
<td>50 (2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>35.5 (0.8)</td>
<td>35.8 (0.8)</td>
<td>ns</td>
<td>21.8 (0.7)*</td>
<td>23.6 (0.9)*</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>43.6 (1.0)</td>
<td>32.5 (1.0)</td>
<td>&lt;0.0001</td>
<td>20.0 (1.5)*</td>
<td>12.7 (1.7)*</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.87 (0.01)</td>
<td>1.00 (0.01)</td>
<td>&lt;0.0001</td>
<td>0.79 (0.01)*</td>
<td>0.88 (0.02)*</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>110 (2)</td>
<td>121 (2)</td>
<td>0.0004</td>
<td>74 (2)*</td>
<td>89 (3)*</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>126 (2)</td>
<td>121 (2)</td>
<td>ns</td>
<td>94 (3)*</td>
<td>101 (1)*</td>
</tr>
<tr>
<td>Total-cholesterol (mmol/l)</td>
<td>5.7 (0.2)</td>
<td>6.1 (0.2)</td>
<td>ns</td>
<td>5.3 (0.3)</td>
<td>6.0 (0.2)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.3 (0.1)</td>
<td>1.1 (0.1)</td>
<td>0.026</td>
<td>1.8 (0.2)*</td>
<td>1.7 (0.2)*</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.6 (0.2)</td>
<td>4.1 (0.2)</td>
<td>ns</td>
<td>3.0 (0.3)</td>
<td>3.9 (0.4)</td>
</tr>
<tr>
<td>Fp-triglyceride (mmol/l)</td>
<td>1.7 (0.1)</td>
<td>2.1 (0.2)</td>
<td>0.093</td>
<td>1.0 (0.1)*</td>
<td>1.0 (0.2)*</td>
</tr>
<tr>
<td>Fp-free fatty acids (mmol/l)</td>
<td>0.72 (0.05)a</td>
<td>0.61 (0.05)b</td>
<td>ns</td>
<td>0.58 (0.05)</td>
<td>0.42 (0.07)*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.4 (0.4)</td>
<td>3.7 (0.6)</td>
<td>ns</td>
<td>0.4 (0.1)*</td>
<td>0.7 (0.1)*</td>
</tr>
<tr>
<td>Fp-glucose (mmol/l)</td>
<td>6.8 (0.4)</td>
<td>7.2 (0.7)</td>
<td>ns</td>
<td>4.9 (0.2)*</td>
<td>5.1 (0.3)*</td>
</tr>
<tr>
<td>Fp-C-peptide (pmol/l)</td>
<td>947 (67)</td>
<td>1020 (91)</td>
<td>ns</td>
<td>353 (27)*</td>
<td>419 (38)*</td>
</tr>
<tr>
<td>Fp-insulin (pmol/l)</td>
<td>80 (8)</td>
<td>83 (11)</td>
<td>ns</td>
<td>13 (2)*</td>
<td>23 (2)*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.3 (0.2)</td>
<td>7.3 (0.5)</td>
<td>0.078</td>
<td>5.6 (0.2)*</td>
<td>5.8 (0.1)*</td>
</tr>
</tbody>
</table>

Data are mean±s.e.m.; P, ANOVA for comparison of obese men and obese women; *P < 0.05 for comparison with obese women; †P < 0.05 for comparison with lean men; ‡P < 0.05 for comparison with lean women; §P < 0.05 for comparison with lean women; ¶P < 0.05 for comparison with lean women.

### Table 2 Intramyocellular triglyceride, anthropometry and insulin sensitivity according to glycaemic control in obese women and lean women.

<table>
<thead>
<tr>
<th>Women</th>
<th>Lean NFG</th>
<th>Obese NFG</th>
<th>Obese IFG</th>
<th>Obese DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (S.E.M.)</td>
<td>Mean (S.E.M.)</td>
<td>Mean (S.E.M.)</td>
<td>Mean (S.E.M.)</td>
</tr>
<tr>
<td>Number</td>
<td>7</td>
<td>13</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>IMTG (mg/g wet weight)</td>
<td>10.9 (2.7)</td>
<td>22.0 (3.9)</td>
<td>18.5 (4.3)</td>
<td>24.9 (3.8)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.8 (0.7)</td>
<td>35.2 (1.2)</td>
<td>34.7 (1.3)</td>
<td>36.7 (1.6)</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>20.0 (1.5)</td>
<td>43.7 (1.6)</td>
<td>43.9 (2.2)</td>
<td>43.1 (1.1)</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.79 (0.01)</td>
<td>0.87 (0.02)</td>
<td>0.86 (0.02)</td>
<td>0.89 (0.01)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.6 (0.4)</td>
<td>5.8 (0.2)</td>
<td>5.7 (1.0)</td>
<td>7.9 (0.9)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.4 (0.1)</td>
<td>2.7 (0.5)</td>
<td>2.9 (0.4)</td>
<td>5.6 (0.9)</td>
</tr>
<tr>
<td>Fp-insulin (pmol/l)</td>
<td>13 (2)</td>
<td>80 (13)</td>
<td>71 (10)</td>
<td>100 (15)</td>
</tr>
</tbody>
</table>

Data are mean±s.e.m.; P, ANOVA. NFG, normal fasting plasma glucose < 6.1 mmol/l; IFG, impaired fasting plasma glucose 6.1–6.9 mmol/l; DM, type 2 diabetes mellitus fasting plasma glucose > 6.9 mmol/l; IMTG, intramyocellular triglyceride; HbA1c, glycosylated haemoglobin; HOMA-IR, homeostasis model assessment insulin resistance index; Fp, fasting plasma.

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available (17). FFA was significantly correlated with IMTG content ($r = 0.43$, $P < 0.005$, Fig. 3). In women, this association remained significant ($r = 0.43$, $P < 0.05$) but not in men ($r = 0.28$, $P = ns$). Neither plasma total cholesterol, HDL-cholesterol nor plasma triglyceride correlated with IMTG content ($P = ns$, data not shown). Of note, the characteristics of this sub-sample of obese subjects, from whom FFA measurements were available, did not differ from the characteristics of the whole group of obese subjects as presented in Table 1.

In a multiple linear regression model including all subjects ($n = 59$) with IMTG as the depending factor and by applying PBF and sex as explanatory variables both explanatory variables could independently explain a total of 27% ($R^2 = 0.27$, $P < 0.001$) of the variation in IMTG (body fat: $\beta = 0.34$, $P = 0.011$; sex: $\beta = 0.27$, $P = 0.041$). In a similar model including subjects with FFA measurements ($n = 44$) and applying PBF and FFA as explanatory variables they explained independently a total of 28% ($R^2 = 0.28$, $P = 0.0012$) of the variation in IMTG (body fat: $\beta = 0.34$, $P = 0.025$; FFA: $\beta = 0.28$, $P = 0.068$).

**Discussion**

The major finding in this study was that obese women possessed twice as much IMTG as compared to age and BMI matched obese men. Moreover, given obesity and insulin resistance, impaired glucose homeostasis was not associated with any further increase in IMTG in obese women or in obese men. Increased insulin, FFA, and PBF were all associated with greater IMTG in women, a finding that could not be reproduced in men in this study. Importantly, it was shown that female gender independently of PBF was associated with increased IMTG.

This study extends previous findings that lean women exhibit greater IMTG than lean men (4, 16) by suggesting, in addition, a gender effect on IMTG in obesity. The mechanisms behind raised IMTG in obese women are thought to involve increased availability of FFA and increased insulin levels (24), which was also suggested by the present results. Cellular transport of plasma FFA is facilitated by fatty acid transport proteins integrated in the plasma membrane of muscle (25). Insulin promotes FFA uptake in cells, and in addition, *in vitro* evidence suggests that FFA uptake in myocytes from obese subjects are increased (26). The present data support, at least in obese women, that deterioration in glycaemic control from a state of NFG, through IFG to type 2 diabetes was not associated with a further increase in IMTG. But the difference in IMTG content was evident between lean women and obese women, both of which subgroups displayed NFG and a similar and normal HbA1c level but a highly different fasting insulin level. Thus, increased IMTG in obese women may be considered an early pathophysiological phenomenon or a physiological adaptation, which may ensue before deterioration in glycaemic control is...
Figure 3 Correlation between free fatty acids (FFA) and intramyocellular triglyceride (IMTG) in a subset of n=44 subjects from whom FFA measurements were available, i.e. n=24 women (17 obese subjects) and n=20 men (15 obese subjects). Circles indicate women and triangles indicate men, lean subjects are indicated by filled marks and obese subjects by open marks. The strength of the correlations is indicated and correlation lines given by a solid line for the total population and by a dashed line for women.

The present findings indicate that the degree of obesity in women may be associated with IMTG level. However, the association suggests that merely 15% of the variation in IMTG may be explained by the level of body fat in obese women, who spanned in BMI from 28.5 to 43.0 kg/m² and in PBF from 35.7 to 58.6%. Recent evidence suggests that extramyocellular triglyceride deposits are associated with obesity in female gender (27). It remains to be investigated whether extramyocellular fat and IMTG content correlate in obese subjects and whether extramyocellular fat releases substances, i.e. adipokines and hormones, which interfere with IMTG turnover. It may be considered that the positive correlation of IMTG versus PBF for the whole group of obese subjects was stronger than for obese women alone as shown on Fig. 2. Thus, while obese men alone had a weak correlation, adding their data clearly improved the overall relationship by extending the range of values. Obese women had a range of body fat values that was approximately double that of men so that may contribute to the higher correlation value for women. This may support that there is not a fundamentally different association between men and women, but rather that obese men and obese women are at different parts of the same curve. However, this alternative interpretation was to some extent contradicted by the results from a multiple regression analysis including all subjects, which revealed that both PBF and sex independently and significantly explained variation of IMTG. This result strongly argues for a gender effect on IMTG content, which is independent of PBF. In addition the data show that for the same level of BMI obese women of gynoid phenotype exhibit significantly more body fat and IMTG than obese men of an android phenotype.

Recent evidence suggests that IMTG by itself may not directly mediate insulin resistance (12). Indeed, by-products of fatty acids not esterified and subsequently deposited as IMTG, i.e. diacylglycerol and ceramides seem to be involved in insulin resistance through inhibition of insulin signalling (11). Obese subjects exhibit increased ceramide level in muscle tissue (28). However, whereas 8 weeks of endurance training decreased ceramide and diacylglycerol concentration in muscle tissue and improved insulin sensitivity, IMTG content did not change (29). Similar observations have been made in the rat model (30, 31). Thus, these data support that lipid intermediates may be a more direct cause of insulin resistance rather than IMTG content per se.

The present study is limited by not examining diacylglycerol and ceramides in muscle, which would likely have provided further mechanistic insight and explanation of the missed or weak associations between the IMTG content versus the glycaemic control level and insulin resistance of obese subjects (11). Lean women have been found to exhibit greater IMTG than lean men (4, 16). Although, this was not reproduced in the present study, it should be emphasized that it was not powered to address this aspect and, accordingly, this was not a part of the predefined endpoints. Had the sample of lean subjects been extended, it might have evolved that lean men had lower IMTG level than obese men, suggesting a similar effect of obesity on IMTG in men as was shown here for women.

It should be recognized that IMTG measurement in muscle may imply an intra-individual coefficient of variation (CV) of 24%, but lower CV should be obtained provided there are relatively large biopsies as in the present study (1). We did not micro-dissect biopsies to remove extramyocellular fat, however, visible fat was carefully removed before freezing of biopsies. Notably, the levels of IMTG content in the present study were similar to prior results obtained in studies irrespective of whether these studies used a biochemical method, the oil-red staining technique or magnetic resonance spectrometry to quantify IMTG (2, 4, 32–34). Recently, Cui et al. suggested that prior results obtained by magnetic resonance spectrometry may have overestimated IMTG content by about 25% due to technical issues on the voxel size used in these studies (35). However, even such correction on the IMTG content obtained in previous studies renders the presently
observed IMTG concentration of about 1% in lean subjects and obese men and 2% in obese women highly plausible. Recently we did careful micro-dissection of muscle biopsies from obese women with similar amount of adipose tissue as in the present study and found an IMTG concentration of ~3%, and for obese men with type 2 diabetes, an IMTG concentration of ~2% was obtained (Dr Rabøl, personal communication, October 10, 2008). These results make it highly unlikely that the present samples should be contaminated by extramyocellular adipose tissue. The oxidative skeletal muscle fibertype 1, which is more prevalent in trained than sedentary subjects, display greater IMTG content than fibertype 2 (34). Although not investigated in the present study, the likelihood of a skewed distribution of muscle fibertypes in the relatively large muscle biopsies, which were harvested from these sedentary subjects, would be small. The participants were not included after a run in period on a controlled diet, which would have strengthened the design. Given that quality of diet, especially content of saturated fatty acids, may impact on IMTG level (25), the study is limited by not providing such data. However, the value of obtaining diet recalls or even diet self-registration must be questioned in a population who struggle with daily overnutrition.

In summary, the present study demonstrates that obese women possess a twofold greater IMTG level than obese men matched for BMI, age, and insulin resistance. Increased IMTG content could be an early pathophysiological defect or a mere physiological adaptation in feminine obesity, which is observed prior to overt impairment in glycaemic control. The data also provide evidence to suggest that increased IMTG of women is associated with increased body fat, circulating FFA, and insulin concentration.

Declaration of interest
The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Funding
This study was supported by grants from The Danish Hospital Foundation for Medical Research, Region of Copenhagen, The Faroe Islands and Greenland; The Danish Diabetes Association; Bernhard and Marie Klein Foundation; and The A P Møller Foundation for the Advancement of Medical Science.

Acknowledgements
Professor, PhD, Carl-Erik Hoy, Department of Systems Biology, Technical University of Denmark, Lyngby, Denmark, has been a great help in planning and performing this study. Technical support from Grete Pettersen at Department of Systems Biology, Technical University of Denmark, Lyngby, Denmark and Susanne Reimer, Hvidovre University Hospital, Copenhagen, Denmark is acknowledged.

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Received 18 February 2009
Accepted 28 April 2009