Increased intramuscular lipid synthesis and low saturation relate to insulin sensitivity in endurance-trained athletes

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Bergman BC, Perreault L, Hunerdosse DM, Koehler MC, Samek AM, Eckel RH. Increased intramuscular lipid synthesis and low saturation relate to insulin sensitivity in endurance-trained athletes. J Appl Physiol 108: 1134–1141, 2010. First published March 18, 2010; doi:10.1152/japplphysiol.00684.2009.—Intramuscular triglyceride (IMTG) has received considerable attention as a potential mechanism promoting insulin resistance. Endurance-trained athletes have high amounts of IMTG but are insulin sensitive, suggesting IMTG content alone does not change insulin action. Recent data suggest increased muscle lipid synthesis protects against fat-induced insulin resistance. We hypothesized that rates of IMTG synthesis at rest would be increased in athletes compared with controls. Eleven sedentary men and 11 endurance-trained male cyclists participated in this study. An intravenous glucose tolerance test was performed to assess insulin action. After 3 days of dietary control and an overnight fast, [13C16]palmitate was infused at 0.0174 μmol·kg⁻¹·min⁻¹ for 4 h, followed by a muscle biopsy to measure isotope incorporation into IMTG and diacylglycerol. Compared with controls, athletes were twice as insulin sensitive (P = 0.004) and had a significantly greater resting IMTG concentration (athletes: 20.4 ± 1.6 μg IMTG/mg dry wt, controls: 14.5 ± 1.8 μg IMTG/mg dry wt, P = 0.04) and IMTG fractional synthesis rate (athletes: 1.56 ± 0.37%/h, controls: 0.61 ± 0.15%/h, P = 0.03). Stearoyl-CoA desaturase 1 mRNA expression (P = 0.02) and protein content (P = 0.03) were also significantly greater in athletes. Diacylglycerol, but not IMTG, saturation was significantly less in athletes compared with controls (P = 0.002). These data indicate endurance-trained athletes have increased synthesis rates of skeletal muscle IMTG and decreased saturation of skeletal muscle diacylglycerol. Increased synthesis rates are not due to recovery from exercise and are likely adaptations to chronic endurance exercise training.

intramuscular triglyceride; isotope; training; exercise; diacylglycerol

INTRAMUSCULAR TRIGLYCERIDE (IMTG) appears to be an important predictor of diabetes risk, since it is inversely related to insulin sensitivity in almost all populations studied (39). In contrast, endurance-trained athletes have a high concentration of IMTG but are very insulin sensitive, which has been termed the “athlete’s paradox” (15). This observation has led to speculation that rates of intramuscular lipid synthesis, rather than concentration, may be related to improved insulin sensitivity (15, 46). Currently, there are no reports in the literature comparing IMTG synthesis in endurance-trained subjects.

There is growing evidence suggesting the ability to increase rates of IMTG synthesis and concentration is associated with insulin sensitivity (22, 31, 32, 49). Protection from fat-induced insulin resistance has been reported with increased IMTG synthesis in muscle cell culture (31), with transgenic mouse models increasing IMTG synthesis or inhibiting degradation (22, 32), and following a single exercise bout in humans (49). Increased synthesis rates of IMTG were associated with decreased ceramide and diacylglycerol (DAG) concentration (32, 49). These data suggest IMTG may act as a sink into which free fatty acids (FFA) are stored, protecting against insulin resistance during increased FFA uptake.

Skeletal muscle DAG is an intermediate of IMTG and phospholipid synthesis and degradation that is increased in insulin resistance (27, 35). Elevated DAG concentration can promote protein kinase C-ε and -θ activity and inhibit insulin signaling (36). The effect of endurance training on muscle DAG concentration is unclear, since both decreased (10) and unchanged (6) concentrations have been reported. Interestingly, there was a trend for decreased saturated lipid content of DAG after endurance training that increased insulin sensitivity in obese subjects (6). We previously reported that insulin-resistant chronic smokers had increased DAG saturation compared with nonsmokers (3). Therefore, data are accumulating suggesting intramuscular DAG saturation may relate to insulin sensitivity. We sought to evaluate whether DAG concentration and composition could explain changes in insulin sensitivity in highly trained endurance athletes. We performed this study to test the hypothesis that resting IMTG synthesis rates are greater and that IMTG and DAG saturations are lower in endurance-trained athletes compared with sedentary control subjects.

METHODS

Subjects. Eleven healthy sedentary men and 11 endurance-trained male cyclists were recruited for this study (Table 1). Subjects gave informed consent and were excluded if they smoked, had diabetes, hyperlipidemia, or liver, kidney, thyroid, or lung disease, or were taking medications that affect glucose or lipid metabolism. Subjects were considered sedentary if they engaged in moderate to vigorous exercise <2 h/wk. The endurance-trained athletes in this study were professional (n = 6), category 1 (n = 1), or category 2 (n = 4) bicycle racers according to the United States Cycling Federation. Subjects were excluded if they had a body mass index <20 kg/m² or >25 kg/m². Subjects were weight stable in the 6 mo preceding participation in this research study. This study was approved by Institutional Review Boards at the University of Colorado Denver and Boulder.

Preliminary testing. After a 12-h overnight fast, subjects reported to the General Clinical Research Center (GCRC), where they were given a health and physical exam and subjected to a fasting blood draw. Body composition was determined using dual-energy X-ray absorptiometry (DEXA) analysis (Lunar DPX-iQ; Lunar, Madison, WI). Insulin sensitivity was determined using an insulin-modified frequently sampled intravenous glucose tolerance test according to standard methods (4). The data were input into MINMOD (Millennium version; Los Angeles, CA) to derive the parameter of insulin action (Si). On a separate day, and following 48 h of recovery from...
the last exercise bout, endurance-trained subjects performed a maximal oxygen consumption (VO_{2 max}) test on an electronically braked cycle ergometer (Lode Excalibur; Quinton Instruments, Seattle, WA). Respiratory gases were measured via indirect calorimetry (respiratory mass spectrometer: Perkin Elmer MGA-1100, St Louis, MO; pneumotachograph: Hans Rudolph Series 3813, Kansas City, MO).

**Diet and exercise control.** All subjects were given a prescribed diet for 3 days before admission to the GCRC. Daily caloric requirement was determined from the DEXA measurement of fat-free mass (FFM) and from analysis of dietary records as previously published (16). The composition of this diet was 55% carbohydrate, 30% fat, and 15% protein. Energy intake and macronutrient composition were not significantly different during the 3-day diet control compared with habitual diet evaluated with a dietary record. The fat content of the diet was controlled with the composition of saturated, monounsaturated, and polyunsaturated fat in a 1:1:1 ratio. Subjects were asked to refrain from exercising for 48 h before the tracer infusion study.

**Tracer infusion study.** After an overnight fast, subjects arrived at the GCRC at 7:00 AM. Blood samples were then taken for determination of body fat-calcium enrichment using the heated hand technique (7). A continuous infusion of [13C6]palmitate (Isotec, Miamisburg, OH) was then initiated at 0.0174 µmol·kg⁻¹·min⁻¹ and continued throughout the study. Indirect calorimetry was performed to measure CO₂ production (VCO₂) at 210 min into the infusion. Blood samples for hormones and substrates were obtained every 10 min from 210 to 240 min of the infusion. A percutaneous needle biopsy was performed following blood sampling after 240 min of isotope infusion for determination of IMTG and DAG concentration, composition, and analysis (3). The muscle biopsies were taken from the last exercise bout, endurance-trained subjects performed a maximal oxygen consumption (VO_{2 max}) test on an electronically braked cycle ergometer (Lode Excalibur; Quinton Instruments, Seattle, WA). Respiratory gases were measured via indirect calorimetry (respiratory mass spectrometer: Perkin Elmer MGA-1100, St Louis, MO; pneumotachograph: Hans Rudolph Series 3813, Kansas City, MO).

**Metabolite and hormone analyses.** Standard enzymatic assays were used to measure glucose and triglycerides (Olympus AU400e chemistry analyzer; Olympus America, Center Valley, PA), lactate (kit no. 826; Sigma, St Louis, MO), and FFA (NEFA kit: Wako Chemicals, Richmond, VA). Insulin was measured using a radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX).

**Muscle lipid analysis.** Skeletal muscle lipid extraction, isolation, and analysis were performed as previously described by our laboratory (3). Briefly, skeletal muscle samples were dissected free of extramuscular fat on ice, lyophilized, added to 1 ml of iced methanol along with internal standards of triptenadecanoic acid and dipentadienoic acid, and homogenized (Omni TH; Omni International, Marietta, GA). Total lipids were extracted (45) and then added to solid-phase extraction columns (Supelclean LC-NH2, 3 ml; Supelco Analytical) to isolate FFAs, phospholipids, IMTG, and DAG. The FFA, phospholipid, IMTG, and DAG fractions were converted to fatty acid methyl esters (FAME), and the stable isotope ratios of 13C in FAMEs were measured using a gas chromatography-combustion isotope ratio mass spectrometer (GC/IRMS) system (Thermo Electron, Bremen, Germany). Concentration and composition analysis was performed on an HP 6890 GC with a 30-m DB-23 capillary column, connected to a HP 5973 MS. Peak identities were determined by retention time and mass spectra compared with standards of known composition.

**Western blotting.** Frozen skeletal muscle samples were weighed and homogenized on ice using a Kontes glass homogenizer (Kimble/ Kontes, Vineland, NJ) in buffer as previously described (3). Protein was extracted, concentration was measured (Calbiochem, San Diego, CA), and 40 µg of sample and internal standard were run on an SDS-PAGE 8% Bis-Tris gel (Invitrogen, Carlsbad, CA) using standard methods as previously described (3). The rabbit anti-human stearoyl-CoA desaturase 1 (anti-hSCD1) was purchased from Alpha Diagnostics (San Antonio, TX), peroxisome proliferator-activated receptor (PPAR)-α and -γ were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and rabbit anti-β-actin was purchased from Cell Signaling (Danvers, MA). Secondary antibodies were obtained from Bio-Rad (Hercules, CA).

**RT-PCR.** Total RNA was extracted from homogenized muscle tissue using the RNeasy mini kit (Qiagen, Valencia, CA). RNA was analyzed and quantified using the Experion system (Bio-Rad). Reverse transcription was performed using 45 ng of total RNA with the iScript cDNA synthesis kit (Bio-Rad). Quantitative PCR was performed using primer sets for genes of interest or two reference genes (all spanned exon-exon boundaries) and ABsolute Blue QPCR SYBR Green fluorescent mix (Thermo Fisher Scientific, Waltham, MA) following the manufacturer’s protocol (Table 2). Reactions were run in duplicate on an iQ5 real-time PCR detection system (Bio-Rad) along with a no-template control per gene. RNA expression data were normalized to levels of ribosomal protein L13a and ubiquitin C using the comparative threshold cycle method.

**Plasma palmitate analysis.** Methylation and extraction of plasma palmitate were performed as described (40). Samples were...
run on an HP 6890 GC with a 30-m DB-23 capillary column, connected to a HP 5973 MS. Enrichments were calculated based on a standard curve of known enrichments and corrected for variations in abundance (41). Peak identities were determined by retention time and mass spectra compared with standards of known composition.

Calculations. IMTG fractional synthesis rate was calculated as previously described for use with stable isotopes in humans, using plasma palmitate as the precursor pool from which IMTG is synthesized (47):

\[
\text{IMTG FSR}(\%) = \frac{E_{\text{IMTG palm}}(t_1) - E_{\text{IMTG palm}}(t_0)}{E_{\text{plasma palm}}(t_1) - E_{\text{plasma palm}}(t_0)} \times \frac{1}{4} \times 100
\]

where \( E_{\text{IMTG palm}}(t_1) \) and \( E_{\text{plasma palm}}(t_1) \) are enrichments of palmitate in IMTG and plasma after 4 h of infusion and \( E_{\text{IMTG palm}}(t_0) \) and \( E_{\text{plasma palm}}(t_0) \) are enrichments of background palmitate in IMTG and plasma, respectively. Background enrichment of IMTG palmitate was determined using enrichment of stearate as previously described, eliminating the need for a baseline biopsy specifically for this purpose (17):

\[
\% \text{Saturation of IMTG and DAG} = \frac{\text{laurate} + \text{myristate} + \text{palmitate} + \text{stearate}}{\sum \text{FFA species}} \times 100
\]

where FFA represents the concentration of individual FFA species in IMTG and DAG after transmethylation.

Statistics. Data are means ± SE. Differences in normally distributed data between controls and athletes were analyzed using one-way ANOVA (SPSS, Chicago, IL). Nonnormally distributed data were log-transformed before analysis using one-way ANOVA. Correlation analysis was performed using the Pearson product moment correlation. An \( \alpha \) level of 0.05 was used throughout.

RESULTS

Subject characteristics and demographic information are shown in Table 1. All subjects were college-age men, although athletes were statistically older (\( P = 0.01 \)). Both groups had a similar body mass index; however, athletes had significantly less body fat (\( P < 0.001 \)) and a lower fasting insulin concentration (\( P = 0.03 \)). There were no differences in fasting plasma glucose, FFA, or triglyceride concentrations. As expected, insulin sensitivity (SI) was significantly greater in athletes compared with controls (Table 1, \( P = 0.004 \)). \( V_{\text{O}_2\text{max}} \) testing was not performed on sedentary controls, but they self-reported <2 h of planned physical activity per week. Athletes had been training 5.8 ± 1.6 yr and were training 14.8 ± 1.4 h/wk during this period of testing. They had \( V_{\text{O}_2\text{max}} \) (68.3 ± 2.1 ml·kg\(^{-1}·\text{min}^{-1} \)) and lactate threshold values (86.8 ± 0.9% \( V_{\text{O}_2\text{max}} \)) indicating a highly trained state (8).

Plasma palmitate enrichment during the last 30 min of the 4-h infusion in both groups is shown in Fig. 1. The values were stable over time and were not significantly different (\( P = 0.18 \)) between endurance-trained athletes and control subjects.

IMTG concentration was significantly greater in endurance-trained athletes compared with controls (Fig. 2A, \( P = 0.04 \)). Increased IMTG concentration was paralleled by a significantly greater fractional synthesis rate (FSR) of the IMTG pool in athletes compared with controls (Fig. 2B, \( P = 0.03 \)). The concentration of skeletal muscle DAG was not significantly different between groups (Table 1). Although there were differences in individual IMTG and phospholipid species, overall the percent saturations of IMTG and phospholipids were not significantly different between groups (Fig. 3, A and C). However, the overall percent saturation of DAG was significantly decreased in endurance-trained athletes compared with control subjects (Fig. 3B, \( P = 0.002 \)). The correlation between SI and DAG percent saturation was statistically significant (\( R = 0.47, P = 0.04 \), Fig. 3D).

Data from RT-PCR analysis are shown in Fig. 4. We evaluated whether there were differences in the expression of...
genes involved in desaturation of long-chain acyl (LCA)-CoA (SCD1), IMTG synthesis (diacylglycerol acyltransferase 1; DGAT1), IMTG degradation (adipose triglyceride lipase; ATGL), nuclear transcription factors controlling expression of genes for mitochondrial fatty acid oxidation and β-oxidation (PPAR-α) and fatty acid synthesis (PPAR-γ), and a transcription factor regulating genes controlling lipogenesis (sterol regulatory element binding protein 1c; SREBP1c). The relative

Fig. 3. IMTG (A), diacylglycerol (DAG; B), and phospholipid (C) %saturation and composition in endurance-trained athletes and control subjects after an overnight fast at rest. D: significant relationship between DAG %saturation and insulin sensitivity (Si). Values are means ± SE. $P < 0.05$, significantly different from control subjects.

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**A** IMTG % Saturation in Controls and Athletes

**B** Skeletal Muscle DAG % Saturation in Controls and Athletes

**C** Skeletal Muscle Phospholipid % Saturation in Controls and Athletes

**D** Relationship between skeletal muscle DAG saturation and insulin sensitivity

\[ R = 0.47 \]
\[ p = 0.042 \]
expression of SCD1 mRNA was significantly greater in athletes compared with control subjects \((P < 0.01)\). There were no differences in relative mRNA expression of DGAT1, ATGL, and SREBP1c mRNA. PPAR-\(\alpha\) \((P < 0.0001)\) and PPAR-\(\gamma\) \((P = 0.0002)\) mRNA expression were significantly greater in athletes compared with control subjects.

Similar to mRNA expression, protein content of SCD1 was also significantly increased in athletes compared with control subjects \((P = 0.03, \text{Fig. 5A})\). However, we found no differences in PPAR-\(\gamma\) \(\text{controls: 1.0} \pm 0.07\ \text{arbitrary units (AU), athletes: 0.96} \pm 0.04\ \text{AU,} P = 0.68\) or PPAR-\(\alpha\) \(\text{controls: 1.0} \pm 0.15\ \text{AU, athletes: 0.81} \pm 0.16\ \text{AU,} P = 0.47\) protein content between groups.

The proportion of energy expenditure from whole body fat oxidation was determined using the respiratory exchange ratio and was not significantly different between groups \((0.81 \pm 0.02, \text{athletes: 0.80} \pm 0.02, P = 0.63)\). Correlations between Si and IMTG FSR \((R^2 = 0.07, P = 0.25)\) were not significant.

**DISCUSSION**

There is a growing body of literature suggesting increased rates of IMTG synthesis protect against insulin resistance during fat-induced insulin resistance in cell culture \((31)\), rodents \((22, 32)\), and humans \((49)\). The historical view of IMTG negatively influencing insulin sensitivity is changing to a new view suggesting the ability to increase IMTG synthesis can prevent insulin resistance. These are the first published data comparing IMTG synthesis rates in endurance-trained athletes compared with those in sedentary subjects. There are two main findings from this study: 1) endurance-trained athletes have significantly increased synthesis rates of IMTG compared with sedentary control subjects, and 2) saturation of the intramuscular DAG pool is decreased in athletes compared with control subjects.

As expected, athletes in the current study were significantly more insulin sensitive and had greater IMTG content compared with controls. It has been hypothesized \((15, 46)\) that increased flux through IMTG may be one mechanism by which athletes are “protected” from the negative relationship between IMTG content and insulin action observed in other populations \((39)\). Our data corroborate this hypothesis and imply that IMTG synthesis, rather than concentration, may be an important link between intramuscular lipids and insulin action \((15)\). After an exercise bout that reversed fatty acid-induced insulin resistance, Schenk and Horowitz \((49)\) reported DAG concentration decreased and IMTG storage increased. These data suggested increased rates of IMTG synthesis prevented accumulation of skeletal muscle DAG. However, differences in insulin action in the current study cannot be explained by differences in DAG concentration, which was not different between groups. Our working hypothesis is that increased neutral lipid synthesis rates may enhance clearance of DAG into synthetic pathways \((\text{i.e., IMTG})\), decreasing interaction with protein kinase C (PKC) and insulin resistance \((44)\). Interestingly, other investigators increased flux through IMTG synthesis in skeletal muscle, via either DGAT1 overexpression in mice \((32)\) or a single bout of exercise \((49)\), and reported protection against fat-induced insulin resistance. These data, combined with our data showing increased IMTG FSR in endurance-trained athletes, advance the idea that high rates of IMTG synthesis may be beneficial to insulin sensitivity. Therefore, increased rates of muscle lipid synthesis in endurance-trained athletes may be one mechanism that helps explain the “athlete’s paradox.”
The current data showing increased IMTG FSR in athletes are different from reports in rodent literature where increased IMTG synthesis rates are associated with insulin resistance in skeletal muscle (21) and myocardium (38). It is possible that these differences are due to species effects in the regulation of IMTG. It also is possible that increased synthesis of IMTG occurs as a function of increased IMTG pool size. More data need to be published, specifically from insulin-resistant subjects, to determine the relationship between IMTG FSR and insulin sensitivity in humans. Our data suggest increased synthesis rates of the IMTG pool are associated with insulin sensitivity in humans.

We measured plasma insulin and FFA concentration, which inhibit IMTG FSR (11, 19, 20, 56), to determine whether regulation by these factors could explain differences in IMTG synthesis rates. There were no significant differences in the concentration of FFA between endurance-trained athletes and sedentary controls. We found a statistically lower fasting insulin concentration in athletes compared with controls; however, it is unlikely that such a small difference in concentration would alter IMTG flux considering the hyperinsulinemia required to decrease IMTG synthesis (20). Recovery from exercise also may influence IMTG use (29). Therefore, we asked subjects to refrain from exercise for 48 h before the study. These data suggest increased synthesis rates of IMTG are a result of chronic exercise training, and not an acute effect of recovery from exercise or alterations in known hormones or substrates that regulate IMTG synthesis.

In addition to intramuscular lipid content, composition of these lipids also may influence insulin sensitivity (30, 33). Alteration in hepatic lipid composition, with increased ratios of 16:1/16:0 and 18:1/18:0, without a change in steatosis, increased hepatic insulin action in a rodent model (33), highlighting the potential importance of lipid composition on insulin sensitivity. Furthermore, insulin-sensitizing lifestyle behaviors such as weight loss and exercise training have been reported to decrease saturation of LCA-CoA, ceramide, and DAG in obese humans (6, 24). Similar to previous reports (6), we did not find a significant difference in overall IMTG saturation between groups. However, overall DAG saturation was lower in athletes compared with controls. In addition, we found a significant inverse relationship between percent saturation of DAG and insulin sensitivity ($P = 0.04$). It is well established that diets rich in saturated fats decrease insulin sensitivity (52, 53). Therefore, it is possible that saturated lipid species may exert a negative influence on insulin sensitivity in skeletal muscle. The literature is unclear on the influence of DAG saturation on PKC activity, which is the leading mechanism explaining DAG-induced insulin resistance (27). PKC activation in vitro has been reported to be higher with unsaturated acyl chains on DAG (23, 48) or independent of saturation (28). However, palmitate administration in primary muscle cell culture increased palmitate incorporation into DAG and resulted in greater PKC activation (35). Our data suggest saturation of skeletal muscle DAG, even without changes in concentration, may be important in modulating insulin action in skeletal muscle after chronic endurance exercise training. Lower intramuscular DAG saturation in athletes does not appear to be due to differences in dietary lipid composition. Measuring dietary fat intake is unreliable by dietary record (50), and therefore previous studies used the overall composition of muscle phospholipids as a surrogate measure of dietary lipid composition (1), which was not different between groups. Because there was no difference in IMTG saturation but a significant decrease in DAG saturation between groups, these data suggest that exercise training specifically changes the composition of intramuscular DAG in skeletal muscle.

Alterations in DAG composition in athletes may be due to increased enzymatic lipid desaturation. One such enzyme is SCD1, which converts saturated palmitoyl-CoA and stearoyl-CoA to monounsaturated palmitoleoyl-CoA and oleoyl-CoA, respectively. Studies reported SCD1 inhibition resulted in an obesity-resistant and insulin-sensitive phenotype in mice (37), and SCD1 content was elevated in obese subjects, which was related to decreased fat oxidation and increased fat storage (25). However, others reported SCD1 content was related to insulin sensitivity in humans (42), and increasing SCD1 content transiently in rat muscle cells protects against fat-induced insulin resistance (43). The definitive answer on the role of SCD1 content in insulin sensitivity is not clear. Based on our data and those of others (26), it appears chronic endurance exercise training increases SCD1 mRNA expression and protein content compared with controls. Increased SCD1 content in athletes may be one mechanism influencing decreased storage of saturated DAG in skeletal muscle.

Other intracellular lipids, including LCA-CoA (12) and ceramide in some (54, 55) but not all studies (51), have been implicated in muscle insulin resistance. Endurance training has been reported to decrease muscle ceramide concentration in rodents (9) and obese humans (6, 10), in addition to decreasing saturated ceramide species (6) in some, but not all, studies (51). The effect of endurance training on LCA-CoA is unclear. Therefore, although not measured in this study, it is possible that changes in LCA-CoA and ceramide content or composition also may influence insulin sensitivity in endurance-trained athletes.

There are several limitations to this study. We did not measure differences in muscle fiber type between subjects, so it is possible that muscle fiber type influenced our results. Palmitate was used as a tracer in this experiment, because this FFA has been previously reported to measure IMTG FSR (18, 47). It is possible that differences in IMTG FSR may be attained using an alternatively labeled FFA; however, previous studies have not found a difference between IMTG FSR measured with oleate and palmitate in rodent muscle (18). We used plasma palmitate as the precursor pool from which IMTG

### Table 3. Stable isotope enrichments for skeletal muscle and plasma lipid species

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Athletes</th>
</tr>
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<tbody>
<tr>
<td>IMTG stearate enrichment (AP)</td>
<td>1.081 ± 0.0012</td>
<td>1.077 ± 0.0018</td>
</tr>
<tr>
<td>DAG stearate enrichment (AP)</td>
<td>1.086 ± 0.0003</td>
<td>1.082 ± 0.0004</td>
</tr>
<tr>
<td>IMTG palmitate enrichment (AP)</td>
<td>1.107 ± 0.0005</td>
<td>1.147 ± 0.008*</td>
</tr>
<tr>
<td>DAG palmitate enrichment (AP)</td>
<td>1.151 ± 0.013</td>
<td>1.190 ± 0.008*</td>
</tr>
<tr>
<td>Plasma TG-palmitate enrichment (MPE)</td>
<td>0.24 ± 0.02</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>Plasma FFA-palmitate enrichment (MPE)</td>
<td>1.10 ± 0.13</td>
<td>1.32 ± 0.14</td>
</tr>
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Values are means ± SE. *P < 0.05, significant difference between controls and athletes. AP, atom percent from gas chromatography-combustion isotope ratio mass spectrometry (GC/IRMS) reflects the 13C enrichment of all carbons in the lipid fraction of interest; MPE, mole percent excess from GC/MS reflects enrichment above background of the M +16 isopomer of palmitate in TG and FFA fractions.
is synthesized in the calculation of IMTG FSR. Plasma FFA and intramuscular FFA enrichment have been used in previous studies; therefore the synthesis rates calculated are similar to some, but not all, published data (3, 17, 21, 47). We used the enrichment of plasma palmitate during the final 30 min of isotope infusion as the enrichment of the precursor pool for IMTG synthesis. However, during a long infusion such as this, plasma triglyceride also becomes labeled with \([^{13}C_{16}]palmitate\), which increases with time (Table 3). Our calculations for IMTG synthesis do not include the contribution of labeled palmitate derived from the action of lipoprotein lipase on plasma triglyceride. We also cannot account for substrate cycling of labeled palmitate, where palmitate derived from IMTG degradation was reincorporated back into IMTG. Our calculations also assume linearity of triglyceride synthesis over 4 h and that no stearate was synthesized during the infusion by elongation of unlabeled palmitate with labeled acetyl-CoA and/or labeled palmitate with unlabeled or labeled acetyl-CoA. Our methods assume that there was no hydrolysis of muscle lipids during microdissection and no contamination of intramuscular triglyceride by extracellular triglyceride during sample processing. Finally, we used a whole body measure of insulin sensitivity, but it is likely that muscle lipid synthesis is more related to muscle insulin sensitivity, which may have decreased our ability to determine such a relationship.

To conclude, these are the first data in humans comparing rates of intramuscular triglyceride synthesis between endurance-trained and untrained individuals. These data are compelling but need to be verified in the future. Our data suggest endurance-trained athletes have significantly increased rates of IMTG synthesis, as well as concentration, compared with sedentary subjects. Furthermore, endurance-trained athletes have a similar DAG concentration to that of sedentary controls. Diacylglycerol saturation was decreased in athletes compared with sedentary subjects, which was significantly related to insulin sensitivity. These data indicate intramuscular lipid synthesis rates and/or DAG saturation may influence insulin sensitivity in endurance-trained athletes.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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2. Bergman BC, Perreault L, Hunerdosse DM, Koehler MC, Samek AM, Nalsen C, Tengblad S, Vessby B. No conflicts of interest, financial or otherwise, are declared by the author(s).


