More insights into a human adipose tissue GPAT activity assay

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ABSTRACT
Adipose tissue fatty acid storage varies according to sex, adipose tissue depot and degree of fat gain. However, the mechanism(s) for these variations is not completely understood. We recently published findings based on the glycerol 3-phosphate acyltransferase (GPAT) enzyme activity assay we optimized for use with human adipose tissue. These findings include a decrease in total GPAT and GPAT1 as a function of adipocyte size in both omental and subcutaneous adipose tissue and a strong, positive correlations between ACS, GPAT, and DGAT activities for both sexes and depots and between these storage factors and palmitate storage rates into TAG. The aim of this commentary is to expand upon the data from our recent publication. We describe here additional details on the optimization of the GPAT enzyme activity assay, a correlation between DGAT and percentage palmitate in the diacylglycerol fraction, and sex differences in fatty acid storage factors and storage rates into TAG at high palmitate concentrations.

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Introduction
We developed a reliable glycerol 3-phosphate acyltransferase (GPAT) enzyme activity assay for human adipose tissue with the aim of determining if GPAT enzyme activity varies by depot, by sex and by adipocyte size in a manner that might account for some of the variations we’ve observed in adipose tissue fatty acid storage rates. We also wanted to delineate if GPAT variations accounted for previously observed variations in adipose tissue fatty acid storage rates.

While GPAT is responsible for the first committed step of triacylglycerol (TAG) synthesis, diacylglycerol acetyl transferase (DGAT) is responsible for the final step of TAG synthesis: the conversion of diacylglycerols (DG) to TAG. We have reported that DGAT activity correlates with rates of FFA storage in subcutaneous fat in some depots under some conditions. In our recent publication we found that DGAT is positively correlated with other fatty acid storage factors: ACS, GPAT, and CD36 as well as palmitate storage rates. Here we expand upon those findings by describing the correlation of DGAT activity to the percentage of palmitate in the DG fraction.

GPAT Assay Development

Adipose whole tissue extract (WTE) yields greater GPAT activity than an adipose tissue pellet

Because we previously found that DGAT enzyme activity was not different between adipocyte mitochondrial/microsomal pellet fraction and adipose WTE, and because of the simpler preparation of a WTE sample, we tested whether there are differences in GPAT activity between these 2 sample preparation approaches. In contrast with our findings for DGAT activity, GPAT activity per mg tissue was significantly greater when the assay was performed using WTE than when back-calculated using the mitochondrial/microsomal pellet from the same samples. Total omental GPAT activity was ~3-fold greater and GPAT1 activity was ~2-fold greater using WTE than using the pellet results (p < 0.05) (Fig. 1A). Likewise, total subcutaneous GPAT and GPAT1 activity calculated using WTE were ~2.5-fold and 3-fold greater, respectively, than when using the mitochondrial/microsomal pellet (p < 0.05) (Fig. 1A). Because WTE consistently displayed significantly greater (p < 0.05) total GPAT and GPAT1 activity than the mitochondrial/microsomal
pellet in both adipose tissue depots (Fig. 1A) we elected to present GPAT activities using the WTE preparation results.

**DGAT activity and accumulation of palmitate in DG**

Omental DGAT activity was negatively correlated with the percentage of palmitate tracer present in the DG fraction for both males \((r = -0.69, p = 0.01)\) and females \((r = -0.68, p = 0.01)\) (Fig. 2A) under conditions of high palmitate concentrations. There was also a significant negative correlation \((r = -0.57, p = 0.04)\) between DGAT activity in omental fat from females and the percentage palmitate in DG at low palmitate concentrations. The correlation between these 2 variables did not reach statistical significance for males \((r = -0.49, p = 0.07)\) (Fig. 2B).

There was no correlation between DGAT activity and the percentage of palmitate in the DG fraction in abdominal subcutaneous adipose tissue at either high or low palmitate conditions for either males or females. \((r\ values = -0.13 \text{ to } -0.55)\) (Fig. 2C–D).

**Sex differences in fatty acid storage factors and storage rates into TAG at high palmitate concentrations**

Palmitate storage rates into adipocyte TAG was significantly greater in women than men relative to ACS \((p = 0.009)\) or DGAT activity \((p = 0.03)\) (Fig. 3A–B). In contrast, there was no significant sex difference in the relationship between palmitate storage rates into TAG and omental adipose tissue total GPAT or GPAT1 activity.

**Discussion**

Our findings that GPAT enzyme activity assays performed with WTE yielded higher GPAT activity than those utilizing the microsomal pellet were unexpected. There are a number of potential explanations as to why we might find greater GPAT activity in the WTE than the microsomal pellet. For example, it is possible that during the centrifugation we employed to isolate the microsomal pellet GPAT is lost from the pellet. Another explanation is that cytosolic proteins or cofactors influence GPAT activity and are lost during the purification step. This was suggested by Saggerson and Rider, who found that at low palmitoyl-CoA concentrations, adding the cytosol to the pellet resulted in a lower GPAT activity compared to the activity measured in the pellet alone, while at high concentrations GPAT activity was greater. We could not disregard our finding that GPAT activity was greater in the WTE than the microsomal pellet, which suggests that measuring GPAT activity in the WTE might be more consistent with the maximal in vivo activity.

We observed a negative correlation between omental DGAT activity and the percentage of palmitate in the DG fraction (Fig. 2A–B), there was no such relationship for abdominal subcutaneous tissue (Fig. 2C–D). Previous studies have shown that DGAT activity is increased in the omental adipose tissue when compared to the subcutaneous depot and there is a positive correlation between DGAT activity and palmitate storage rates in the omental depot but not the subcutaneous. Our results build on these previous reports by suggesting that sufficiently high omental DGAT activity can more efficiently convert DG to TAG and thus avoid high intracellular DG concentrations. These results suggest that individuals with lower relative DGAT activity have an accumulation of FFA in adipocyte DG and this accumulation of FFA in the DG fraction could contribute to insulin resistance in omental fat.

Interestingly, there was a sex difference between the relationships between ACS and DGAT activity and palmitate storage rates into TAG in omental tissue (Fig. 3A–B), but not a sex difference in the GPAT/palmitate storage rate relationship. However, GPAT relative to ACS and DGAT activity is greater for females than males. These results are consistent with our previous findings that women have greater palmitate storage into adipose tissue than men.4 The development of a reliable
Figure 2. Correlation between DGAT and percentage palmitate in the diglyceride (DG) fraction. (A–D) Solid circles, females; Open squares, males. (A) Omental tissue, high palmitate concentration: relationship between DGAT activity (pmol TG/mg lipid/min) and palmitate storage rates into DG fraction (\(\mu\)mol/min/mg lipid) for women \(r = -0.68, p = 0.01\) and for men \(r = -0.69, p = 0.01\). (B) Omental tissue, low palmitate concentration: relationship between DGAT activity (pmol TG/mg lipid/min) and palmitate storage rates into DG fraction (\(\mu\)mol/min/mg lipid) for women \(r = -0.57, p = 0.04\) and for men \(r = -0.49, p = 0.07\). (C) Abdominal subcutaneous tissue, high palmitate concentration: relationship between DGAT activity (pmol TG/mg lipid/min) and palmitate storage rates into DG fraction (\(\mu\)mol/min/mg lipid) for women \(r = -0.41, p = 0.18\) and for men \(r = -0.55, p = 0.06\). (D) Abdominal subcutaneous tissue, low palmitate concentration: relationship between DGAT activity (pmol TG/mg lipid/min) and palmitate storage rates into DG fraction (\(\mu\)mol/min/mg lipid) for women \(r = -0.13, p = 0.68\) and for men \(r = -0.14, p = 0.65\).

Figure 3. Sex differences in fatty acid storage factors and storage rates into TAG at high palmitate concentrations. (A–B) Solid circles, females; Open squares, males. Square root-transformed values were used to achieve normal distribution. (A) Relationship between ACS activity (pmol/min/mg lipid) and palmitate storage rates (\(\mu\)mol/min/mg lipid) for women \(r = 0.77, p = 0.0007\) and for men \(r = 0.80, p = 0.0006\). (B) Relationship between DGAT activity (pmol TG/mg lipid/min) and palmitate storage rates (\(\mu\)mol/min/mg lipid) for women \(r = 0.70, p = 0.006\) and for men \(r = 0.82, p = 0.0003\).
GPAT enzyme activity assay has allowed us to define variations in GPAT activity among adipose tissue depots, sexes, and ranges of adipocyte size. Future investigation of GPAT and other fatty acid storage factor activity under varying metabolic states may prove useful in delineating differences in fat distribution and progression to metabolic disease states.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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