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Revisiting the diacylglycerol-induced insulin resistance hypothesis

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Summary

Obesity is associated with skeletal muscle insulin resistance, which is a crucial step in the development of type 2 diabetes. Among the mechanisms by which obesity may lead to insulin resistance, lipotoxicity is one of the hypotheses being explored; others include inflammation or the oxidative stress hypotheses. This review focuses on the role of diacylglycerols (DAG), a family of lipid metabolites implicated in the pathogenesis of lipotoxicity and insulin resistance. While recent studies report contradictory results in humans with regards to the importance of DAG-induced insulin resistance in skeletal muscle, other current literature highlight a potential role for DAG as signaling molecules. This review will discuss possible hypotheses explaining these contradictory results and the need to explore further the role of DAG in human metabolism.

Introduction

Obesity has become a major health care problem in the developed countries. In the United States, the incidence of obesity has increased from 14% to 23% to 30% in each of the past decades [1]. Diabetes and the metabolic syndrome have followed the same pattern [2, 3]. In 2011, about 25.6 million persons had diabetes, which corresponded to an estimate of 11.3 % of the US adult population [4].

Insulin resistance (IR) is known as a characteristic trait of type 2 diabetes mellitus (T2DM) [5]. The worsening of insulin action is a continuum beginning with peripheral IR and ending with a loss of insulin secretion [6]. IR involves defects in multiple organ systems such as the skeletal muscle, the liver and the pancreas [7]. Due to its relative anatomical importance (30-40% of body mass), skeletal muscle accounts for approximately 80% of the insulin-stimulated glucose uptake [8, 9]. Skeletal muscle IR can be present and precedes for many years the onset of T2DM [7, 10]. IR is one of the principal mechanisms by which obesity is considered to increase the risk of T2DM and is a key feature of the metabolic syndrome.

Lipotoxicity is one of the hypotheses being explored to explain the mechanisms by which obesity leads to IR. Other theories include the inflammation or the oxidative stress. Lipotoxicity, also known as the lipid metabolite theory, occurs when fatty acid in excess of the oxidative needs spillover into harmful pathways of nonoxidative metabolism [11].

Within the muscle fibers, excess accumulation of triglycerides (intramyocellular lipids, IMCL) has been associated with skeletal muscle IR [12-14]. Numerous human studies have confirmed the inverse association between IMCL and insulin sensitivity [15-18]. However, this inverse association between IMCL and IR is not observed in endurance exercise training and/or conditions for efficient fatty acid utilization. Chronic endurance exercise has been shown to increase IMCL in parallel with improved IR [19, 20], thus leading to a paradigm, known as the 'athlete paradox' where IMCL accumulation per se does not directly affect insulin action. Thus the deleterious effect of increased

IMCL appears to be linked with the non-utilization of the fatty acid reservoir and with the accumulation of metabolically active lipid intermediates [21]. Among the lipid intermediates that have been identified as potentially playing an important role in mediating fatty acid induced IR in muscle, there are four main families: diacylglycerols (DAG) [22, 23], ceramides [24-26], long chain fatty acyl-CoAs [27-29] and acylcarnitines [30-33].

This review will focus solely on DAG-induced IR. We will discuss recent studies that have shown contradictory results pointing out the need to revisit the role of DAG in IR. We will also briefly review other studies that have underscore the importance of DAG as signaling molecules. Finally, we will consider possible reasons for the contradictory results about DAG-induced IR in human skeletal muscle and discuss alternative hypotheses on the role of DAG in skeletal muscle metabolism.

Overview of DAG structure and mechanisms of DAG-induced insulin resistance

DAG can be produced from triacylglycerol (TAG) hydrolysis, from phospholipids hydrolysis or de novo synthesized from monoacylglycerol (MAG) [34]. Their structure and localization depends on their origin. Indeed 1,3-DAG and 2,3-DAG derive from TAG lipolysis and are mostly in lipid droplets, while 1,2-DAG come from esterification and accumulate mostly in the membranes; *de novo* synthesized DAG from phosphaditic acid hydrolysis is present in the endoplasmic reticulum [35]. **Figure 1** shows the chemical structures of TAG and DAG molecules.

Studies pointing to the potential mechanisms involved in DAG-induced insulin resistance derive from *in vitro* and animal research [36, 37]. The proposed mechanism is that DAG activates serine/threonine kinases C (PKC) isoforms [38, 39] that in turn decrease tyrosine phosphorylation of the insulin receptor substrate 1 (IRS-1) and as a consequence decrease PI3-Kinase activation [40, 41]. Thus, the common view, largely based on evidence from cell systems and animal models, is that muscular DAG content explains IR in obesity and T2DM [42].

Not all DAG are equal, indeed 1980's early works described 1,2-DAG as the only active stereoisomer capable of activating PKCs [43], while 1,3 and 2,3-DAG [44] showed virtually no activity. Other studies pointed to an extreme level of specificity [45], for example the introduction of a single methyl group into the diglyceride backbone produced a DAG with virtually no kinase activating ability [46]. Finally, the importance of the chain length and saturation into the activity of the DAG is also apparent from early biochemical works. Ebeling et al. [47] presented that DAG with saturated acyl side chains were as effective as those with unsaturated chains in kinase activation. Other groups supported that having two saturated fatty acids was less effective that one unsaturated fatty acid at any position, irrespective of chain length [48]. Studies investigating chain length were dependent on the models used, indeed although some groups showed that different chain lengths were equipotent at interacting with the regulatory site *in vitro*, such as diC18:1, diC10:0 and diC8:0, chains of 10 carbons or greater prevented the interaction of DAG with their regulatory site on the kinase in intact cells, mostly due to cell permeability [47]. This high degree of specificity for structural features contrasted, with that observed for phospholipid bilayers or mixed micelles, where all lengths of acylchains appeared to be active [45]. It is important to note that none of this biochemical work has been done in skeletal muscle cells, thus that the specific roles of fatty acids chain lengths and degree of saturation in DAG induced muscle IR have not yet been elucidated.

Association between human muscle DAG content and insulin resistance

In 2002, the first study in humans pointing to the positive association between DAG content and IR was a lipid infusion intervention [38]. In this study, Itani et al. performed, in six healthy middle-age males volunteers, lipid infusions combined with a 6 hours euglycemic-hyperinsulinemic clamp, that is the gold standard method to measure insulin sensitivity *in vivo* [49]. A control group of 6 other volunteers received the insulin infusion without lipids. *Vastus lateralis* biopsies were performed at baseline, at 2 hours and at 6 hours of the infusion. Infusion of lipids and insulin resulted in a three-

fold increase in total DAG content after 6 hours. This was concomitant of an increase in PKCs activity. Insulin sensitivity was reduced in the lipid infusion group compared to the control group.

Since then, numerous studies performed with human muscle have confirmed the positive relationship between total DAG content and insulin resistance. These are presented in **Table 1**. In 2007, Schenk et al. [50] performed a randomized cross-over study in healthy women to investigate the effect of acute exercise combined with lipid infusion on muscle DAG and IR measured by intravenous glucose tolerance test (IVGTT). They hypothesized that a single session of exercise in human subjects would protect against fatty acid–induced insulin resistance the next day. Indeed, when subjects received the lipid infusion and were sedentary, their total muscle DAG content was increased. When they received their lipid infusion and exercised, exercise prevented DAG increase and the reduction in insulin sensitivity.

Cross-sectional studies also confirmed the relationship between muscle DAG content and IR. In 2007, Straczkowski et al. [51] observed that total muscle DAG were higher in obese (with and without impaired glucose tolerance) than lean controls and lean offsprings of type 2 diabetics. Total DAG were inversely related to insulin sensitivity measured by glucose clamps. Later Moro et al. [52] compared sedentary subjects with a broad range of BMI and age, including type 2 diabetics. They observed that intramyocellular DAG content was elevated in obese vs. non obese subjects. They further looked at the fatty acid profile and found that DAG containing oleic acid (18:1) were higher in the obese than the non-obese volunteers. Insulin sensitivity was negatively correlated with DAG. DAG content was not correlated with age, BMI, or body fat. Recently, Bergman et al. [53] compared intramyocellular DAG from obese diabetic men, obese non-diabetic men and women and lean trained athletes. Total DAG content was higher in diabetics than the two other groups; and was negatively correlated with insulin sensitivity measured by IVGTT. Using ultracentrifugation, they fractionated DAG species in cytosolic vs. membrane DAG. Approximately 80% of DAG were localized in membranes, these were lower in athletes compared to obese diabetics and non-diabetics.

Cytosolic DAG were lower in obese compared to diabetics and athletes. While membrane DAG content was negatively correlated in insulin sensitivity, cytosolic DAG content was not correlated with insulin sensitivity.

Dubé et al. conducted two intervention studies in overweight or obese sedentary men and women [54, 55]. The first one in 2008 [54] was a moderate aerobic exercise intervention. In a pre/post design, study volunteers underwent muscle biopsies and clamps before and after a 16 weeks supervised exercise training. Muscle DAG content was decreased with exercise training in conjunction with improved insulin sensitivity. The second intervention conducted in 2011 [55] was a 16 weeks randomized controlled study with two arms: diet induced weight loss (DIWL) or exercise. The DIWL was geared to achieve 10% of weight reduction through approximately 500 kcal deficit per day. The exercise intervention was similar to the 2008 study. Both groups improved their insulin sensitivity by approximately 20% and decreased DAG content within skeletal muscle. No significant correlation was observed between the change in DAG content and the change in insulin sensitivity. Due to the fact that DIWL decreased total IMCL content while exercise increased IMCL, this study suggested a repartitioning from non-esterified fatty acids away from DAG into neutral lipids stores with exercise or a decrease in total lipid content with diet induced weight loss.

Dissociations between human muscle DAG content and insulin resistance

In 2006, Bruce et al. [56] did the first study to investigate the effect of endurance training on muscle DAG in humans. They observed no difference in intramyocellular DAG content before and after 8 weeks of aerobic exercise in nine obese subjects. **Table 2** lists the human studies that point to a negative or no association between DAG content and IR.

Lipid infusion studies also presented conflicting results. Vistisen et al. [57] performed biopsies before, during glucose clamps with or without lipid infusion and after 30 minutes acute bout of exercise at the end of the clamps. They observed reductions in insulin sensitivity with the lipid

infusion but no changes in muscle DAG content neither before nor after the exercise bout. Recently, Hoeg et al. [58] performed glucose clamps after five hours of lipid infusion or saline infusion to healthy volunteers. Muscle biopsies were performed at baseline, after the five hours infusions and during the glucose clamps. Insulin sensitivity decreased approximately 25-35% after lipid infusion compared to saline infusion. No difference was observed in muscle DAG content in the lipid infusion compared with the control trial, nor a decreased in the insulin-signaling pathway. Taken together, these studies suggest that lipid oversupply decreases whole body insulin sensitivity without changes in intramyocellular DAG content, thus challenging this currently accepted mechanism for acute lipid-induced insulin resistance.

Numerous cross-sectional studies have not been able to show differences in muscle DAG across groups of distinct insulin sensitivity levels. Anastasiou et al. [59] compared obese type 2 diabetics with non diabetics. Diabetics had higher HOMA-IR and higher IMCL content but no difference in muscle DAG content compared to non-diabetics. Similarly Perreault et al. [60] compared obese with impaired glucose tolerance to obese with normal glucose tolerance. No group differences were found in DAG concentration or DAG percent saturation. In a nested case-control study, Coen et al. [61] compared insulin sensitive to insulin resistant sedentary obese women. No differences were observed in total DAG content, in specific moieties or in degree of saturation. There was no correlation between intramyocellular DAG and insulin sensitivity. In a sub-cohort of men with metabolic syndrome, Van Hees et al. [62] categorized those with lower insulin sensitivity and those with higher insulin sensitivity assessed by OGTT. Their muscle content did not differ in total DAG content in the fasting state or after a high fat meal. Differences in the degree of saturation or specific DAG moieties were however found and are discussed below.

When comparing obese to lean women, Trush et al. [63] observed no difference in total DAG, total saturated or total polyunsaturated moieties. Monounsaturated DAG were higher in the lean vs. obese, which was mostly explained by an increase in palmitoleic acid. Jocken et al. [64] compared

obese to lean men. They observed lower DAG content in muscle from obese compared to lean. This was also true for saturated, monounsaturated and polyunsaturated fatty acid species in DAG, which were lower by more then 30% in the obese men. To our knowledge, this is the first study to describe higher DAG in muscle from lean non-sedentary men as these subjects were included if they had less than three hours of organized sport activities [65]. Compatible with this observation, we found total DAG content to be ~50% higher in muscle from chronically endurance trained subjects (5 or more exercise sessions per week) than lean sedentary subjects (exercising < 1 day per week, < 20 minutes), who in turn had ~20% higher content than obese sedentary subjects matched by age [66]. Similar differences were observed for saturated DAG and for DAG species in which one of the fatty acids was unsaturated. This same pattern was also true for insulin sensitivity, with higher insulin sensitivity in trained individuals compared to lean sedentary, which in turn were more insulin-sensitive than obese sedentary subjects. Total DAG was positively correlated with insulin sensitivity. In a cross-sectional study comparing professional cyclists to healthy men (exercising < 2 hours/week of moderate or vigorous intensity), Bergman et al. [67] found no difference in muscle DAG content between. These studies suggest that a higher DAG content is not necessarily related to IR, and they are in accord with an animal study showing that increased DAG content in muscle is not related to IR [68].

Some intervention geared at reducing insulin resistance in type 2 diabetes also reported dissociations between improvements in insulin resistance while observing no changes in DAG. In a pre/post-intervention design, Anastasiou et al. [69] followed a group of obese diabetic women before and after a 10% weight loss reduction through hypocaloric diet. Although total intramyocellular TAG decreased, they observed no significant differences in total DAG, saturated or unsaturated DAG. Bajaj et al. [70] performed muscle biopsies and glucose clamps before and after four months of pioglitazone in diabetic subjects. Although they observed a decrease in insulin resistance, total DAG did not change after the intervention.

Of particular interest is the role of exercise training, indeed in chronically endurance trained subjects, total DAG content was higher in athletes and DAG content was positively associated with insulin sensitivity [66]. In another cross-sectional study, overall DAG content was not different in cyclists compared with healthy volunteers that were less insulin sensitive than the cyclists [67]. Although acute and chronic exercise have distinct mechanisms, acute exercise in context of lipid oversupply protected against the increase in DAG [50, 57].

Taken together, these studies point to the fact that total DAG content is not necessarily related to IR; alternatively, it is possible that particular DAG species may be associated with IR.

Specific moieties of DAG

Distinct molecular species of DAG within muscle have been explored in a subset of human studies (Tables 1 and 2). Some studies point to differences in fatty acids (FA) chain lengths [55, 56] or degree of FA saturation [62, 67], but these results show important variability across the studies. When looking into fatty acid profile, Van Hees et al. [62] indicated that insulin resistant men presented higher percentage of palmitic acid (C16:0) and lower oleic acid (C18:1) than insulin sensitive subjects matched by weight. Moro et al. [52] noticed a significant increase in DAG containing oleic acid (C18:1) in obese vs. non obese subjects. Thrush et al. [63] showed that lean women had more palmitoleic acid (C16:1) and decosahexaenoic acid (C22:6) compared to obese women.

Three recent studies [53, 55, 66] point to the fact that some DAG moieties are particularly abundant in human skeletal muscle: C16:0/C18:0, C16:0/C18:1 and diC18:0. Taken together, these three DAG species accounted for approximately 80% of total DAG [66]. Although statistical significance across groups or in relationship with insulin sensitivity differ among these studies, the data suggests that it is not the overall content of DAG that may be deleterious but that particular DAG moieties, even in smaller amounts, may confer the lipotoxic effect.

Another similarity in the two cross-sectional studies with insulin sensitive athletes compared to insulin resistant obese or T2DM [53, 66] is that DAG species that contained two unsaturated fatty acid were more abundant in obese or T2DM compared to highly insulin sensitive athletes. Athletes had higher content of saturated DAG and DAG species in which only one of the two fatty acids was unsaturated compared to their insulin resistance counterpart.

One of the possible explanations for the important variability of these results is that habitual diet composition influences FA composition. Some studies requested their volunteers to eat a certain proportion of macronutrients in the days preceding the muscle biopsies, other fed their subjects, while others did not control food intake at all. This point is important as it is known from cultured myocytes studies that the exposure to palmitic acid (C:16:0) led to enhanced DAG levels and consequent activation of PKC, in contrast exposure to oleic acid (C18:1) did not [71]. Recently, Krien et al. [72] showed no change in insulin sensitivity nor in DAG content in healthy humans before and after a 7 day diet on either palmitic acid (C16:0) or oleic acid (C18:1). Nevertheless, the incapacity to control for chronic habitual diet in studies investigating the composition of intramyocellular lipids is a main limitation in all of human studies that could in part explain the important variation across studies. Further dietary interventions are needed to explore the impact of diet on human DAG-induced IR.

Another important limitation in the comparison of these studies is related to the methodology used to measure DAG. Some studies only measured DAG mass (synonym to total DAG content), others measured individual FA after removing the glycerol backbone, while others measured the FA composition without removing the glycerol backbone. Some studies measured the degree of saturation in percentages, others measured the absolute levels of saturated, monounsaturated or polyunsaturated FA. None of these human studies measured the specific positions of the FA-binding to the glycerol backbone (1,2-DAG, 1,3-DAG and 2,3-DAG).

Lipid intermediates are signaling molecules in futile cycles

A possible explanation of the variability of intramyocellular DAG content in the human studies presented above is the role played by DAG as signaling molecules in futile cycles. An illustration of futile cycle is one of the central pathways connecting lipid and glucose metabolism called the glycerolipid/free fatty acid cycle (GL/FFA), also known as the triglyceride/FA cycle [35](Figure 2). This cycle includes a lipolytic arm and lipogenic arm. The lipid synthesis arm starts with FA esterification in fatty-acylCoA which then bonds to a glycerol backbone in the form of glycerol-3-P. The last step in this lipogenesis is the acetylation of 1,2-DAG into TAG. The lipolytic side starts with TAG hydrolysis to 2,3-DAG and ends with the hydrolyzation of 2-MAG into FFA and glycerol. Both of these can be recycled, indeed about half of the FFA released in the lipolytic segment is recycled into the lipogenic segment [73]. At all times, the cells undergo a continuous synthesis and degradation of TAG even in low energy demands situations [74]. This cycle takes place by consecutive actions of specific enzymes that are distributed in the membrane, cytosol, ER, nucleus and lipid droplets in a location-dependent regulation [74]. This cycle is referred as 'futile' as it consumes ATP and produces heat while recycling substrate [75]. The GL/FFA has been demonstrated to exist in cultured muscle cells and to protect from lipid oversupply IR [76]. Indeed, it is hypothesized that certain toxic metabolites are rendered less toxic by conversion to corresponding esters and stored in lipid droplets or transported out of the cells [77].

Another important link between glucose and lipid metabolism is the glucose derived malonyl-CoA which reduces fat oxidation in the mitochondria through its inhibitory action on carnitine palmitoyltransferase I. As a result fatty acyl-CoAs (FACoA) accumulate in the cytoplasm. This FACoA accumulation causes an exaggerated production of various reactive complex lipid-signaling molecules that may lead, among other negative effects, to IR and diabetes. Enhanced GL/FFA cycling activity provides an attractive mechanism by which a cell might escape the toxic action of fuel oversupply for both glucose or lipids oversupply [35]. Other futile cycles linking glucose

metabolism and lipid metabolism may also be involved, in particular the substrate cycle between de novo lipogenesis and lipid oxidation [78], which has been proposed as a mechanism by which leptin protects skeletal muscle from excessive fat storage and lipotixicity [79].

Two key signaling metabolites that are thought to provide a crucial link between intracellular fuel homeostasis and cell signaling processes are DAG and MAG. Among the signaling molecules derived by the GL/FFA cycle, DAG and MAG play multiple roles in the regulation of many pathways depending upon their site of production. To date, the effect of exercise on the GL/FFA cycle, or other substrate cycles linking glucose and lipid metabolism, is not known.

In addition of their role in the insulin signaling cascade by activating PKCs and as signaling molecules in futile cycles as the GL/FFA cycle, DAG also play an important role in exocytosis and neurotransmission [80], as well as inflammation. Indeed, by activating a particular PKC isoform (PKCθ), DAG activate the pro-inflammatory pathway NFκB [39], which is thought to be one of the links between insulin resistance and inflammation [81]. Thus, different DAG stereoisomers and fatty acids content may have different roles regarding these diverse functions of DAG.

Conclusion

Although DAG have been implicated in lipotoxicity in cellular systems and animal studies, recent human studies have yielded controversial findings pertaining to the DAG-induced insulin resistance hypothesis. Even if human data linking DAG to IR is mostly correlative and limited by the lack of consistency across measurement methods and research design (including dietary and exercise control), there are other hypotheses that could explain these conflicting findings.

The first one is that that until now, DAG mass was measured in whole muscle lysates, thus not taking into account the importance of compartmentalization. Future studies need to assess subcellular localization, i.e. cytoplasmic membrane vs. organelle membranes vs. lipid droplets, as

well as DAG structure in terms of position of the fatty acid chains on the glycerol backbone in addition of fatty acid chain length or degree of saturation.

Another possible explanation for the variability and inconsistencies in human studies is that the measurements of DAG content only reflect values at a specific time point, without any information on their fluxes. As discussed above, DAG are intermediates of multiple cellular processes such as the GL/FFA cycle. What we measure at a given time point could be the result of a physiological phenomenon linked with the GL/FFA cycle and not necessarily linked with a toxic effect. Thus, further studies exploring the GL/FFA cycle in human muscle and the modulations of this cycle by chronic exercise seem necessary in order to understand role of DAG as second messengers in energy metabolism pathways.

Taken all of this together, future research in the field of lipotoxicity and particularly DAG-induced IR should explore the effects of dietary interventions on DAG content as well as time-dynamics on the DAG pool consequently of exercise and food intake. Furthermore, it will be important to define subcellular localizations of DAG subpopulations, are these in the membrane, cytosol or in the lipid droplets? In conjunction with the questions asked here, DAG structure need to be described in terms of sterospecific isoforms, chain lengths and degree of saturation. Which DAG are involved with IR, are these 1,3- or 2,3- or 1,2-DAG? What chain lengths in which position and what degree of saturation in which position? Although some of these questions may be responded by animal studies and cell cultures, the heterogeneity and dynamic nature of DAG needs to be explored in human to be able to determine the relative contribution of DAG in human IR.

Figure legend

Figure 1: *TAG and DAG.* Chemical structures of triacylglycerol and diacylglycerols with the hydrocarbon chain of fatty acid esterified on the glycerol backbone. The fatty acyl groups may have different saturation levels (not shown here).

Figure 2: *Glycerol/FFA Cycle* (adapted with permission from Prentki et al. 2011) . Glycerol/FFA cycle is the cyclic process of esterification of FFA into a glycerol, followed by its hydrolysis with the release of the FFA that can be reesterified. GL/FFA allows for continuous production of lipid intermediates that include triacylglycerol (TAG), diacylglycerol (DAG) monoacylglycerol (MAG), plysophosphatidic acid (LPA) and phosphatidic acid (PA). The lipogenic arm is represented with red arrows, the lipolytic arm with green arrows. The lipolytic arm is regulated by specific lipases that include adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL) and MAG lipase (MAGL). Hydrolysis by ATGL and HSL are facilitated by perilipin and comparative gene identification 58 protein (CGI-58).Fatty acyl CoA (FACoA) can either enter the lipogenic arm at various levels or enter beta-oxidation in the mitochondria for beta-oxidation

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Table 1, Human studies showing an association between muscle DAG content and IR

1st author (ref #)	Year	Design (CS or I)	Subjects	N	Intervention	Nutrition controlled	AP controlled	Results	Comments
Itani [38]	2002	I	Healthy males	6	Acute effect of 6h lipid infusion	Y	N	Increase in IR and total DAG	Specific moieties not measured
Straczkowski [51]	2007	CS	All males, 4 groups: A. Healthy lean controls B. Lean offsprings of T2DM C. OW or OB NGT D. OW or OB IGT	A12, B12, C12, D9 (27 for DAG: 9,7,7,5)	NA	N	N	Total DAG D and C > A. No differences between A and B, nor between C and D. Total DAG was inversely related to IS.	No differences in individual DAG.
Schenk [50]	2007	I	Healthy women	8	Acute effect of lipid infusion with or without one exercise bout	Y	N	Total DAG increased with lipid infusion. Exercise prevented IR and DAG increase.	Specific moieties not measured, no clamp.
Dubé [54]	2008	I	OW or OB older sedentary	25 (13 for DAG)	Aerobic exercise, 16 wks, 3 days/wk	Y	Y	Decrease in IR. Decrease in total DAG. No association between change in DAG and change in IS.	Specific moieties not measured
Moro [52]	2009	CS	All sedentary, broad range BMI and age, including 10 T2DM	48	NA	Y	Y	Total DAG higher in OB and T2DM. IS was negatively correlated with DAG.	C18:1 content higher in OB and T2DM.
Dubé [55]	2011	Randomized I	OW or OB older sedentary	16	Diet-induced weight loss with or without exercise, 16 wks, 3 days/wk	Y	Y	Both interventions reduced IR and total DAG. No association between change in DAG and change in IS.	Exercise decreased C14:0/18:0, C16:1/18:0, C16:1/18:1, C18:0/18:1, Di-C16:1, Di-C18:0, Di-C18:1.
Bergman [53]	2012	CS	3 Groups: A: Athletes B: OB C: T2DM	22 (10, 6 and 6)	NA	Y	Y	Total DAG B and C > A, negatively correlated with IS. Cytosolic DAG A and C > B; no correlation with IS. Membrane DAG B and C > A, no correlation with IS.	No clamp. C had higher membrane content of C18:0/C20:4, Di-C16:0 and Di-C18:0.

CS: cross-sectional
I: intervention
N: sample size
Y: yes
N: no or no information available
IR: insulin resistance or insulin resistant
IS: insulin sensitivity or insulin sensitive

OW: overweight
OB: obese
T2DM: type 2 diabetes
NGT: normal glucose tolerance
IGT: impaired glucose tolerance
AP controlled: study controlling for chronic physical activity
Nutrition controlled: study controlling for meal composition (either in intervention or during the days before biopsy)

Table 2, Human studies pointing out for a disconnect between muscle DAG content and IR

1st author (ref #)	Year	Design (CS or I)	Subjects	N	Intervention	Nutrition controlled	AP controlled	Results: DAG content in human skeletal muscle	Comments
Bruce [56]	2006	I	OB sedentary	9	Aerobic exercise, 8 wks, 5 days/wk	Y	Y	No significant decrease in total DAG.	No clamp. Significant decrease in C16:0.
Vistisen [57]	2008	I	OB men and women	16	Acute effect of lipid infusion and acute exercise	Y	Y	Decreased IS after lipid infusion was recovered with acute bout of exercise. No change in total DAG.	Specific moieties not measured.
Anastasiou [59]	2009	CS	OB T2DM vs non T2DM	30 (9 vs. 19)	NA	N	N	No difference in total DAG. No correlation between DAG and IS.	No clamp. Specific moieties not measured
Trush [63]	2009	CS	Lean vs OB women	33 (18 vs 15)	NA	N	N	No difference in total DAG. No differences in sum of saturated DAG or polyunsaturated DAG. Lean had higher content of monounsaturated DAG.	No measure of IS. Individual moieties: Lean had more 22:6, tended to have more 16:1.
Perreault [60]	2010	CS	OB NGT vs IGT	39 (19 vs 20)	NA	Y	N	No difference in total DAG or degree of saturation.	Correlation between DAG and IS not given.
Coen [61]	2010	CS	OB sedentary women, IS vs IR	22 (10 vs 12)	NA	Y	Y	No difference in total or specific DAG. No correlation between DAG and IS.	No differences in specific moieties.
Jocken [64]	2010	CS	Lean vs OB men	23 (13 vs 10)	NA	N	Y	Total DAG Lean> OB. Saturated, monounsaturated and polyunsaturated species of DAG were also lower in OB.	
Bergman [67]	2010	CS	Healthy sedentary vs. cyclists men	24	NA	N	Y	No difference in total DAG. IS cyclists > sedentary.	No clamp. Cyclists had higher 18:1, 18:2, but lower 16:0, 16:1 and 18:0.
Anastasiou [69]	2010	I	OB T2DM sedentary women	5	Hypocaloric diet, 17 to 32 weeks to achieve 10% WL	N	Y at baseline, N during intervention	No change in total DAG, saturated, monounsaturated or polyunsaturated DAG.	No clamp
Bajaj [70]	2010	I	T2DM	10	Pioglitazone for 4 months	Y	Y	No change in total DAG. IR decreased with intervention.	
Van Hees [62]	2011	CS	OW or OB men with metabolic syndrome, IS vs IR	30 (15 vs 15)	NA	N	Y	No difference in total DAG. No differences in DAG content after a high fat meal.	No clamp. Higher degree of saturation in IR men explained by higher C16:0 and lower C18:1*n-9*.
Amati [66]	2011	CS	3 Groups: A: Athletes B: Lean sedentary C: OB sedentary	42 (14, 7 and 21)	NA	Y	Y	Total DAG A > B and C. Same pattern for sum of saturated and unsaturated DAG on one FA. C had higher content of unsaturated DAG on both FA.	Higher in A: C14:0/16:0, C16:0/18:0, C16:0/18:1, C16:1/18:0, C18:0/18:1, Di-C18:0. Higher in C: C16:1/18:1, Di-C14:0, Di-C16:1.
Hoeg [58]	2011	I	Healthy young	16	Acute effect of lipid infusion	Y	Y	No change in total DAG. IS decreased with lipid infusion.	Specific moieties not measured.

CS: cross-sectional
I: intervention
N: sample size
Y: yes
N: no or no information available
IR: insulin resistance or insulin resistant
IS: insulin sensitivity or insulin sensitive
OW: overweight
OB: obese
T2DM: type 2 diabetes
NGT: normal glucose tolerance
IGT: impaired glucose tolerance
AP controlled: study controlling for chronic physical activity
Nutrition controlled: study controlling for meal composition (either in intervention or during the days before biopsy

Figure 1

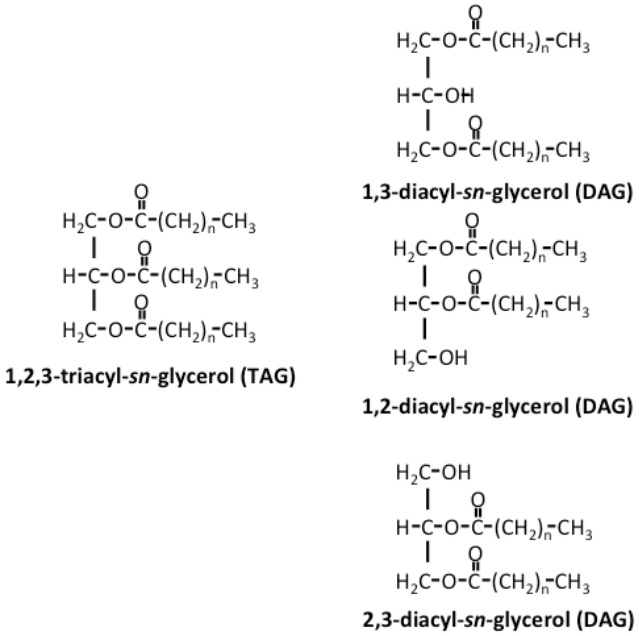
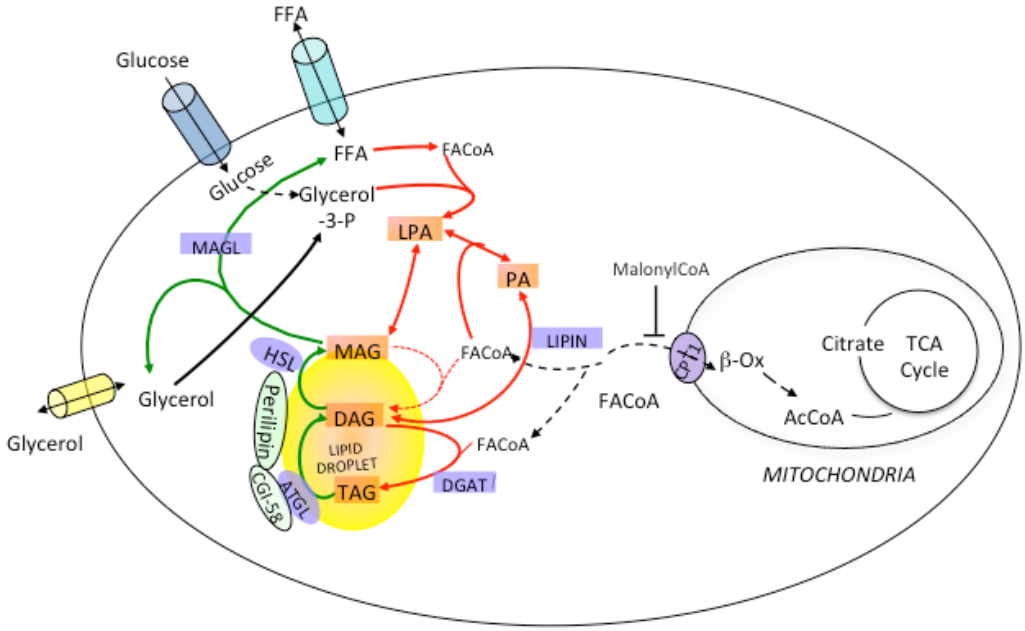


Figure 2



Adapted from Prentki & Madiraju, Mol Cell Biol 2011