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Reassessing the Role of Diacylglycerols in Insulin Resistance

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Abstract

Skeletal muscle (SM) insulin resistance (IR) plays an important role in the burden of obesity, particularly it leads to glucose intolerance and type 2 diabetes. Among the mechanisms thought to link IR to obesity is the accumulation, in muscle cells, of different lipid metabolites. Diacylglycerols (DAG) are subject of particular attention due to reported interactions with the insulin-signaling cascade. Given that SM accounts for the majority of insulin stimulated glucose uptake, this review integrates recent observational and mechanistic works with the sole focus of questioning the role of DAG on SM IR. Particular attention is given to subcellular distributions and specific DAG structures, which epitomize the future direction towards reaching a consensus on the mechanistic role played by DAG.

Diacylglycerol and skeletal muscle insulin resistance: historical perspective in the context of disease

Obesity and type 2 diabetes (T2D) have reached epidemic proportions [1,2]. Insulin resistance (IR) is strongly linked with obesity and is considered the cornerstone of T2D [3,4]. IR damages multiple organs including liver, muscle, visceral adipose tissue and others. At its most severe stage, IR can lead to pancreatic β-cell failure which results in the cessation of insulin production [5,6]. Skeletal muscle (SM) accounts for ~60%–80% of glucose uptake in response to insulin [7]. The contribution of SM IR to whole body IR is supported by a large body of literature [8-11] and by mathematical models [12].

The lipotoxicity theory explains how dysfunctional mitochondria, particularly at the level of the β oxidation machinery, can be overwhelmed by excess of fatty acids (FA) and lead to the accumulation of FA metabolites into non-adipose tissues (*i.e.* ectopic fat) [13,14]. Ectopic fat accumulation is seen as a hallmark of lipotoxicity and, is considered as one of the mechanisms that link obesity with IR [15]. Intramuscular triglyceride (IMTG) accumulation was considered a major player in the development of SM IR in humans [16-19]. The only exception to this notion is the observation that endurance-trained athletes display paradoxically high levels of insulin sensitivity (IS), despite having greater content of IMTG [20,21]. In the past 2 decades, evidence has shown that it is not IMTGs per se that are incriminated in IR, but other bioactive lipid intermediates. While some evidence currently supports ceramides as contributors [22], the involvement of DAG in the development of SM IR is still being investigated. Here we discuss literature published since our initial review on the same topic in 2012 [23]. Particular attention has been given to human data that incorporates the

different stereoisomers and moieties of DAG, their subcellular localization and their relationship with SM IR. Furthermore, animal and cell culture experiments will be discussed to provide further insight into the current status of the field.

DAG structure, pathways and mechanisms

DAG originate either from hydrolysis of triacylglycerol/phospholipids or appear as a lipid intermediate during de novo synthesis of TAG from glycerol-3-phosphate, the building block of all phospholipids [24]. DAG can occur in three different stereoisomers *sn*-DAG 1,3-DAG, 1,2-DAG or 2,3-DAG (figure 1). The stereospecific numbering (*sn*) designates the position of the fatty ester at the glycerol backbone. In addition of these structural isomers, 1,2 and 2,3 DAG have spatial isomerism and are known as enantiomers [25]. For this review, we use the abbreviated form (without sn). Different DAG stereoisomers have distinctive bio-physiological characteristics [26]. By 2012, the common notion was that 1,3 & 2,3 DAG were metabolically inactive [27] and only 1,2 DAG were capable of activating Protein Kinase C (PKC) isoforms [28]. Different stereoisomers of DAG tend to have specific localizations within the cell. TAG lipolysis derived 1,3 and 2,3 DAG are commonly located around LD whereas, FA esterification derived and/or de novo synthesized 1,2 DAG tends to accumulate in membranes [29], *i.e*. in the sarcolemma and intracellular membranes such as at the level of the endoplasmic reticulum and mitochondria.

Besides their stereospecificity, DAG species differ one from another by the different FA species esterified to the glycerol backbone. In this review, we use the terms ‘DAG species’ or ‘DAG moieties’ to designate specific molecules of DAG that differs one from another by their FA chain length or degree of saturation. In the same logic, we

term ‘total DAG’ to nominate the complete set of DAG measured in a sample as opposed to specific DAG isomers or species.

There are multiple systems of naming FA used variably in literature. For this work, we chose to use only lipid numbers to give a uniformed coherence. To ease the comparison with other papers, here is a list of the most frequent FA found in DAG with their lipid numbers and common names: C14:0 (Myristic acid), C16:0 (Palmitic acid), C16:1 (Palmitoleic acid), C18:0 (Stearic acid), C18:1 (Oleic acid), C18:2 (Linoleic acid), C20:4 (Arachidonic acid), C24:0 (Lignoceric acid) and C24:1 (Nervonic acid).

DAG have been incriminated as metabolically toxic due to the activation of PKC isoforms which in turn disrupt the insulin signaling cascade (Box 1) [30-32]. For almost 4 decades, DAG FA degree of saturation and/or carbon chain length were thought to be key components of DAG induced IR [33,34]. Examples encompass studies showing that DAG containing one FA chain of C18:1 were higher in obesity and T2D [35], C16:0 being higher in insulin resistant men [36], or C16:1 and C22:6 higher in lean women [37]. Research in the field has evolved with numerous studies since 2012 investigating the different stereoisomers and moieties of DAG in the context of SM IR.

Human studies showing no association between skeletal muscle DAG and IR (table 1)

Six cross-sectional studies in the past 6 years did not detect association between DAG and IR. Coen and colleagues reported no difference in total SM DAG content

between lean and obese insulin resistant women [38]. De la Maza and colleagues reported total SM DAG to be dissociated from obesity, IR and aging [44]. Sogaard and colleagues reported no association between total or several species of DAG with HOMA-IR in different age and training groups [52]. The same group confirmed no differences in IS between sedentary obese younger and older subjects, while pointing to a higher total content of total DAG and particularly DAG containing one FA chain of distinct saturated species [53]. Similarly to our previous report in chronically endurance trained athletes [54], Perreault and colleagues confirmed the athlete's paradox with high levels of total DAG in highly insulin sensitive athletes and comparable levels of total DAG in humans with T2D which were at the opposite side of the insulin sensitivity scale [51].

Nine intervention studies reported no significant associations between total DAG and IR. Liang et al. found that pharmacological reduction of circulating free FA in obese individuals, with or without T2D, causes improvements in insulin signaling without significant change in total DAG content [39] suggesting that DAG does not play a part in FA mediated lipotoxicity. Although exercise intervention increased IS, Louche et al. reported no change in total DAG in obese men [41]. Sogaard and colleagues found that total and specific DAG species were not different in SM of offspring of patients with diabetes compared with age and gender-matched controls and didn't explain IS improvements in response to endurance training [45]. Chow and colleagues demonstrated similar reduction in IS upon lipid infusion in sedentary or trained subjects, despite increases in total and specific DAG in the sedentary [42]. Using a dual stable-isotope approach to differentiate between metabolic fates of dietary versus endogenous FA, Goossens and colleagues reported that individuals with

impaired glucose tolerance have lower accumulation of DAG despite having lower levels of IS compared with individuals with impaired fasting glucose. The authors conclude that it may not be DAG accumulation per se but rather disturbances in SM FA handling that contribute to IR [46]. Taken together, these studies disconnect improvements or deteriorations in IS from muscle DAG content.

To end this section, it is important to note recent works that looked at DAG stereoisomers. Lundsgaard and colleagues reported that a hypercaloric diet high in unsaturated fat increased 1,3 DAG but reduced IS by down regulation of muscle glucose uptake rather than by interference with insulin signaling [48]. Bak et al. reported no differences in 1,2 and 1,3 DAG in SM of obese and lean individuals, neither at baseline, nor in response to 72h fasting, even though this intervention decreased whole body IS in both groups [49]. In a cross-sectional comparison between lean, endurance-trained athletes, obese with T2D and obese without T2D, Perreault et al. found no differences in 1,3-DAG. Athletes had the highest level of IS and higher total DAG and 1,2 DAG content compared with both lean and obese, similar to levels observed in T2D [51]. These studies suggest that IR can occur without elevation of muscle DAG and that IS can occur with elevation of muscle DAG.

Human studies showing an association between skeletal muscle DAG and IR (table 2): focus on subcellular localization

Since our previous review [60], many studies examined the role of subcellular distribution of DAG. These works will be discussed with three distinct focuses: subcellular localization, DAG moieties and DAG stereoisomers.

A recurring theme in the current literature is that membrane DAG are elevated in individuals with T2D [55,56,58]. Bergman et al. reported that both total and membrane DAG, particularly saturated membrane DAG, are associated with IR in a cross-sectional comparison among sedentary obese controls, individuals with T2D and lean endurance-trained athletes [55]. Jocken et al. and Nowotny et al. both observed a significant positive relationship between membrane DAG and IR [56,57]. Szendroedi and colleagues reported that acute induction of IR through lipid infusion in lean insulin sensitive individuals increases total and membrane DAG content [58], further leading to preferential increments in distinct membrane DAG [57].

The consensus is less evident for cytosolic DAG. Lipid infusion studies show increments in cytosolic DAG [58] while cross-sectional studies show higher [58], lower [55] or similar [56] cytosolic DAG in T2D compared to normal glucose tolerance. Correlations between cytosolic DAG and IS are reported as negative [58] or not significant [51,55].

It is here important to acknowledge that fractionation techniques have evolved fast. Indeed, while studies published in 2010-2014 separated membranes vs. cytosolic DAG, most recent protocols allow separation of different membranes, where specific DAG content can be measured for example in the cell membrane (sarcolemma), mitochondria or endoplasmic reticulum. This fine-tuned ability to measure DAG in different subcellular localizations may explain discrepancies between earlier studies. A good example is the work done by Bergman and Perreault in their successive works. Following their 2012 observation on DAG localization [55], Bergman et al. reported that total DAG content was not different in obese, individuals with T2D and athletes at rest or in response to an acute exercise bout. As they observed higher

IMTG synthesis rate at rest in the athletes, which correlated with higher cytosolic accumulation of DAG and with IS, the authors conclude that chronic endurance exercise promotes high rates of IMTG synthesis, which alters intramuscular lipid localization and may explain the athlete's paradox [50]. Recently, the same authors report that subjects with T2D and endurance-trained athletes have similar and higher amounts of total DAG and sarcolemma DAG compared with obese non-diabetic or lean sedentary volunteers, without differences in cytosolic DAG [51]. Looking at other subcellular compartments, they also observed that total DAG and 1,2 DAG in mitochondria and endoplasmic reticulum, as well as in the nucleus, were higher in the athletes and were positively associated with IS. Overall, these findings highlight the importance of investigating the subcellular localization of the different DAG species. This compartmentalization may, in part, explain the athlete paradox which was considered at its origin bound to lipid droplets and IMTG [21], later involving also specific lipid metabolites such as DAGs [51,54], which, in light of the recent studies using organelle fractionation, seem bound to membranes and specific DAG moieties [51].

Human studies showing an association between skeletal muscle DAG and IR (table 2): focus on DAG moieties and DAG stereoisomers

In the past 6 years, research has also focused on DAG moieties and their specific contribution and association to IR. In supplemental table S1, we combined all observations available in human muscle, ranking DAG moieties by abundance. When quantitative data was not available, abundance was based on figures. Before comparing specific DAG across different studies, it is important to acknowledge

188 methodological issues (tables 1 and 2). While most recent studies used lipidomic
189 techniques, such as liquid chromatography tandem mass spectrometry with or
190 without fragmentation, other studies used thin layer chromatography with or without
191 radiolabeling through kinase assays [61,62]. Importantly, none of these methods
192 have been validated to differentiate DAG enantiomers.

193 As previously observed [54,63], current literature confirms that the most frequent (top
194 three) DAG moieties are Di-C18:0, C16:0-C18:0 and C16:0-C18:1, with some
195 discrepancies among studies. For 3 independent studies the most abundant DAG is
196 C16:0-C18:0 [38,44,55], for 2 reports it is DAG C16:0-C18:1 [43,45] and all others
197 studies have their own champion. Only two studies looked at the different moieties
198 within different compartments and here too abundances varied [55,58].

199 Many species of DAG have been thought to modulate IS but there is no consensus
200 on those that specifically alter IS (table S1). We only found three moieties for which
201 data was confirmed at least in two or three independent studies: Di-C18:2, Di-C14:0
202 and C18:0-C20:4. Di-C18:2 was found to be higher in total DAG of obese insulin
203 resistant [59], in the cytosolic and membrane fractions of T2D and obese [58] and
204 increases were observed after lipid infusion and high fat diet (HFD) [57,58]. Di-C14:0
205 was found higher in obese in one study [54] and correlated with PKCε activation in
206 another study [55]. C18:0-C20:4 was observed to be higher in the membrane fraction
207 of obese and individuals with T2D [55,58] and in the cytosolic fraction [58]. This
208 observation questions the role of FA composition of the diet given the fact that 20:4 is
209 one of the long chain omega-3 polyunsaturated essential FA that cannot be
210 synthesized *de novo* by humans and are thought to play an important role in human
211 health by reducing the risk of chronic diseases [64].

Nine DAG moieties have diverging, or even opposing, results across studies. A good example is C16:0-C18:1 which was observed to be higher in the cytosol of obese and subjects with T2D [58] but was also reported as being higher in athletes [54] and particularly in the membrane fraction of athletes [51]. Yet in another study, membrane C16:0-C18:1 was correlated with PKCε activation without being significantly different in individuals with T2D compared to obese and athletes [55]. Other examples are DAG C18:1-C18:2 and C18:0-C18:2 that were found to increase in response to exercise intervention without improvements in IS [47] or were increased after lipid infusion [58]. Finally, Perreault et al report a positive relationship between PKCε activation and sarcolemma 1,2 DAG C16:0-C18:2 [51].

Taken together, these observations point to the importance of doing a systematic comparison among studies, measurement methods and human variability. We hope that table S1 will be useful in comparing and discussing relevant results with an integrative approach as proposed in the concluding remarks.

DAG and IR: findings from *in vitro* studies (table 3)

In vitro evidence regarding the association between DAG and IR in recent years is also equivocal. Here, we summarize all studies from 2012 to today that report measures of DAG and insulin signaling or IS in SM cell lines or primary myotubes. Importantly, we consciously decided not to incorporate studies using cultures of cardiomyocytes or cardiomyoblasts as these have distinct histological, physiological and metabolic properties than SM cells. We prefer not to risk inferences from these different models.

Two studies using rat L6 myoblasts incubated with palmitate (C16:0) observed impaired insulin signaling, but one found no associated changes in total DAG [70] while the other observed increases in total DAG and specific DAG [68]. When incubating these cells with palmitoleate (C16:1), Pillon and colleagues found a positive association between C16:1- DAG and IS [70].

Two studies using mice C2C12 myoblasts show that FA composition of DAG resembles FA provided in the incubation media. When incubating these cells with palmitate (C16:0), cellular DAG content increased in conjunction with impairments of the insulin signaling cascade [66,67]. Co-incubating palmitate (C16:0) with oleate (C18:1) decreased DAG content, impacted PKCθ phosphorylation and improved insulin signaling in a dose-specific manner [67]. A recent work using the same model presents arguments disconnecting DAG, and specific DAG species, from the insulin-signaling cascade [68]. While studying the positive effect of vitamin D on IS, Jefferson and colleagues found that calcitriol, the active metabolite of vitamin D, increased insulin-stimulation of p-Akt while increasing total DAG and many DAG species [69]. Finally, Bosma and colleagues show a disconnect between DAG and IS in two separate experiments [65]. When incubating C2C12 cells with C16:0 and modulating PLIN2 content by knocking down or overexpression, they observed increases in C16:0- containing DAG without compromising insulin signaling (when they knocked down PLIN2) or improving IS (when overexpressing PLIN2).

DAG and IR: evidence from animal studies (table 4)

Similar to human studies and *in vitro* investigations, *in vivo* animal studies fail to confirm the relationship between DAG and SM IR.

Timmers and colleagues found that treating mice with etomoxir improved insulin signaling and IS despite the accumulation of DAG [71]. Thus, blocking FA from entering the mitochondria and the subsequent accumulation of DAG doesn't necessarily lead to IR. Selathurai et al. observed that very high DAG content, particularly membrane DAG, do not cause IR. Indeed, they created a muscle specific KO mice lacking one of the phospholipids biosynthesis pathways (CDP-ethanolamine pathway) which resulted in a 200% increase in DAG content [79]. Exploring the influence of DAG stereoisomers, Serup and colleagues observed that in hormone sensitive lipase (HSL) KO mice, the accumulation of 1,3 DAG, which originate from adipose triglyceride lipase (ATGL) mediated lipolysis after an acute bout of exercise, don't inhibit insulin stimulated glucose disposal [81]. Taken together these works point to situations were DAG and IS have independent behaviors from one another.

In parallel to their *in vitro* observation mentioned above, Bosma and colleagues modulated PLIN2 expression *in vivo* in rats and observed that improvements in IS may be accompanied with increases in IMTG without modifications in DAG content [65]. Matravadia et al. demonstrated that linoleic acid and α-linoleic acid supplementation mediate glucose homeostasis preservation in obese Zucker rats while at the same time increasing total DAG and many species of DAG [80].

In different mouse strains, Badin et al. [74] and Turner et al. [75] found that HFD supplementation increased total DAG, impaired insulin signaling and contributed to the initial development of IR. Bruce et al. reported that sphingosine-1-phosphate

analog FTY720 treatment in conjunction with HFD, prevents the accumulation of total or specific DAG, and consequently results in improved glucose homeostasis [76].

Zabielski and colleagues, identified specific DAG species that responded to HFD alone and/or to the combination of HFD with metformin in conjunction with the deterioration or amelioration of IS in Zucker rats [82]. Intriguingly some DAG species were elevated in both conditions, while other were decreased. In the same puzzling impression, Turner and colleagues pointed to the relationship between SM specific DAG content and HFD-induced IR in mice, but perplexingly specific DAG varied at different time-points for the same degree of IR [75]. Similarly unclear are the results from Holloway et al. reporting the effects of *in vivo* muscle contraction secondary to unilateral electrical stimulation in obese and lean Zucker rats [77]. When looking at the absolute values reported in the supplemental data, specific DAG moieties were higher or lower depending on the type of muscle measured. With chronic contraction, insulin stimulated glucose uptake increased in both lean and obese, white and red stimulated muscles compared to their contralateral sham controls. While IMTG increased in all muscles, DAG content didn't change significantly in lean muscles nor did it change in white obese. Only in the red muscle from obese rats did chronic contraction change total DAG. Among all the DAG moieties measured, the only significant modifications with chronic contractions were an increase in DAG containing C16:1- and a reduction in C20:0-. Taken together, these works point to the difficulty on identifying specific DAG properties linked with IR even in animal models.

PKC isoforms involved in DAG induced SM IR

Classification and differences among PKC isoforms are described in a separate Box. Despite considerable research, which PKC isoform is the most pertinent in DAG mediated IR remains to be elucidated. This section emphasizes the lack of consensus raised by the current state of investigation. Before expanding on the differences between studies, it is important to acknowledge a potential reporting bias. Indeed, studies not supporting the DAG-induced IR theory do not report PKC data. Out of the five human studies that reported an association between SM DAG and IR, three reported links with PKCθ, one with PKCδ and one with PKCε. Among the six animal studies, only one reported a link with PKC (PKCθ and PKCε).

PKCθ is the most frequent isoform incriminated in altering insulin signaling in SM [83,84]. Yu et al. demonstrated that lipid infusion in Wistar rats increased PKCθ activity and subsequently resulted in increased IRS-1 serine phosphorylation [85]. Similarly, Griffin and colleagues also highlighted the role of PKCθ in the insulin cascade in lipid infused rats [86]. In human muscle, Szendroedi et al. demonstrated that lipid infusion caused IR, increased cytosolic and membrane DAG and temporarily increased PKCθ activity, concomitantly with increases in IRS-1 serine phosphorylation, inhibition of insulin-stimulated IRS-1 tyrosine phosphorylation and AKT2 phosphorylation [58]. They also reported which DAG species had the strongest relationship with PKCθ (table S1). Nowotny and colleagues reported increments in PKCθ activity after oral fat ingestion and after lipid infusion [57], but they observed a significant correlation between PKCθ and overall membrane DAG, and specifically membrane Di-C18:2 DAG, only when fat was ingested orally. Sogaard and colleagues found no differences in PKCθ or p-PKCθ^{ser676} protein expression between

326 insulin sensitive controls and offspring of patients with T2D, neither before nor after
327 exercise training [45].

328 Other PKC isoforms have also been associated with altered insulin signaling in
329 human muscle. Itani et al. found that lipid infusion increases in DAG caused
330 heightened activity in PKCβII and PKCδ [30]. Jocken and colleagues reported data
331 supporting a potential role of increased PKCδ activity in response to increments in
332 saturated membrane DAG [56]. Bergman and colleagues found that four specific
333 cytosolic DAG were negatively related to PKCε but not PKCθ activity. On the other
334 hand, they observed two membrane DAG species that explained a positive
335 relationship to PKCε, but not PKCθ activity (table S1) [55].

336 Here again, it is important to consider intracellular localization and stereoisomers.
337 Empirical evidence has long held the view that 1,2 DAG (as opposed to 1,3 and 2,3
338 DAG) is the sole stereoisomer that can activate PKC [26]. Perreault et al. reported
339 significant positive relationship between PKCε and sarcolemma 1,2 DAG C16:0-
340 C18:2 [51] (table 1). No other localization or DAG moiety was associated with PKCε,
341 PKCθ, PKCδ, or PKCβII.

342 Further to this, both *in vivo* and *in vitro* studies have shown conflicting evidence
343 regarding DAG induced PKC activity in IR. Timmers et al. reported no increments in
344 membrane PKCθ protein content in mouse muscle after Etomoxir treatment [71].
345 Badin et al. reported that DAG mediated IR development in HFD fed mice is
346 positively associated with higher membrane to cytosol ratio of PKCθ and PKCε [74].
347 More recently, Capel and colleagues demonstrated that a treating C2C12 myotubes
348 with oleate can decrease PKCθ activity and improve IS [67].

Overall, based on the aforementioned divergent findings, it is quite clear that the jury is still out on which PKC isoforms are the most pertinent in the context of SM DAG induced IR. Moreover, it probably is the apt moment for lipotoxicity research to reach for some consensus on measurement strategies of PKC isoforms to avoid further confusion about their importance.

Concluding remarks

Whether DAG play a causal role in SM IR in humans is inconclusive. In the past 6 years, lipotoxicity research has moved beyond the acceptance of total DAG as a main culprit of lipid intermediate driven IR in SM. In analyzing and summarizing the data leading up to 2012, it appeared that in all experiments, DAG were measured in whole muscle lysates, thus neglecting the importance of compartmentalization. It is evident that research since then has addressed this issue and advanced the knowledge base around DAG induced IR to a certain extent. Unfortunately, given divergent results in human and animal studies presented in this review, it impossible to make a conclusive judgment on how much weight should DAG receive in the potential etiology and treatment of SM IR or T2D.

Methodological reasons including study design, differences sample collection, age of the study participants, gender differences and genetic variances may have contributed to these divergent findings. Although advancement of lipidomics technologies have greatly helped in advancing the knowledge, analytical expressions are still inconclusive as some report full DAG with the two FA and other report separate FA from glycerol backbone. To ascertain the real impact of DAG on SM IR,

we believe that an integrative point of view which considers stereoisomers and moieties with attention to chain lengths, degree of saturation, and their subcellular localization is needed.

While we focused this review on skeletal muscle, it is possible that other organs, particularly liver or heart, may provide different conclusions. Indeed, the causality of DAG induced hepatic IR seems much more established however was beyond the scope of this review.

Among other perspectives, the role of PLIN proteins in modulating DAG and IMTG are needed. PLIN2 and PLIN5 are considered as main promotors of IMTG storage [87,88]. Latest evidence indicate that having a high IMTG synthesis rate and having lipids partitioned into TAG in LD is a protective feature [50]. Another direction for future research would be to focus on time lapse/dynamic experiments. As it stands at present, most available data are describing snap shots regarding DAG involvement in lipid metabolism and the insulin cascade.

Collectively, despite considerable progress in recent years, many questions remain unanswered and the exact role of DAG in the development of IR is yet to be established. It is possible that DAG are not as influential in SM IR as it once was thought. Investigations encompassing subcellular localizations and comparisons among different DAG moieties as well as DAG stereoisomers will open new perspectives in the field.

Figure legend

Figure 1, DAG stereoisomers (Adapted from Eichmann [25])

395 R_1 and R_2 are fatty acid chains commonly expressed as $CH_3(CH2)_n$

396

397 **Box: Protein Kinase C (PKC) isoforms**

398 PKC are classified into classical, novel and atypical isoforms. [89-91]. Classical (or
399 conventional) PKC isoforms include PKCα, PKCβI, PKCβII and PKCγ. Their
400 activation depend on DAG and calcium [91,92].

401 Novel PKC isoforms include PKCδ, PKCε, PKCη and PKCθ [84]. The main difference
402 between classical and novel PKC is that the novel ones are calcium independent.
403 Novel PKC have a twofold greater affinity for DAG than conventional PKC [93].

404 Atypical PKC isoforms include PKCζ, PKCι and PKCλ. These do not require calcium
405 nor DAG and are activated by 3-phosphoinositide-dependent kinase-1 mediated
406 phosphorylation. In the latter instance, the DAG involved are a byproduct of the
407 process of TAG formation.

408 Lipid oversupply mediated DAG accumulation and subsequent PKC activation
409 provides one of the main mechanistic explanations to the link between intracellular
410 lipid accumulation and the generation of IR [94]. PKC disrupt the insulin signaling
411 cascade via serine and threonine phosphorylation of the insulin receptor, insulin
412 receptor substrate 1 (IRS-1) and potentially other proteins [83].

413 1,2-DAG is a potent activator of PKC isoforms [95]. This stereospecificity links PKC
414 activation to DAG origin, *i.e.* lipolytic vs. lipogenic DAG respectively derived from
415 phospholipase C activity at the plasma membrane or synthesized at the ER
416 membrane as a result of dietary oversupply of lipids [84].

PKC isoforms cross talk in cells and may be key for the functional integration of signaling networks. These cross talks between PKC isoforms contribute to their own activation or inhibition, thus bringing another level of complexity to the already existing methodological challenges including state specific antibodies and assay variations [90].

Box: Glossary

Acipimox: pharmacological compound used to reduce triglyceride levels and increase HDL cholesterol. It is a niacin derivative acting on the niacin receptor 1 and inhibiting the enzyme triglyceride lipase.

Adipose Triglyceride lipase (ATGL) also known as **Patatin-like phospholipase domain-containing protein 2 (PNPLA2)** is one of the major enzyme involved in the intracellular degradation of triglycerides. Catalyzes the initial step in triglyceride hydrolysis in adipocyte and non-adipocyte lipid droplets by interaction with CGI-58

Akt, also known as Protein kinase B (PKB): A serine/threonine specific protein kinase belong to the insulin signaling cascade. The ratio of phosphorylated Akt/Akt (p-Akt/Akt) is used to assess Akt activation.

Diacylglycerol (DAG): consists of a glycerol backbone linked with two fatty acyl chains. DAG can exist in three different stereoisomers.

Enantiomers: represent two stereoisomers that appear each as the mirror image of the other and are not superimposable

Etomoxir: pharmacological compound used to inhibit fat oxidation. It is an irreversible inhibitor of carnitine palmitoyltransferase 1 on the outer mitochondrial membrane. It prevents de formation of acylcarnitines and thus the transport of fatty acids from the cytosol to the mitochondria.

Fatty acid (FA): a carboxylic acid with a long aliphatic chain, which can either be saturated or not (see box 1)

Hyperinsulinic Euglycemic (HE) clamp: method of assessment of insulin sensitivity/insulin resistance. The technique is based on the principle of "clamping" plasma glucose concentration while giving a continuous and constant infusion of insulin and maintaining euglycemia with a variable dose of exogenous glucose infusion. The assumption is that at steady state, the rate of glucose infused is equal to the rate of glucose uptaken. This can be combined with the use of stable isotope glucose tracer to evaluate endogenous hepatic glucose production.

HOMA-IR: A mathematical model to assess insulin resistance called the homeostatic model. Using fasting glucose and insulin, it allows to estimate β-cell insulin secretion and insulin resistance.

Intramyocellular lipids (IMCL): general term for fat stored in muscle cells, often used as synonym for lipid droplets.

Intra muscular triglyceride (IMTG): the part of IMCL that is made of triglyceride. It is usually measured by immunohistochemistry using Oil-Red-O that stains neutral lipids. It is considered the most abundant form of lipid within muscle.

Insulin resistance (IR): Impaired ability of insulin to display its physiological effect on targeted tissues (SM, fat, liver) usually due to an alteration of insulin signal

transduction within the cell. Insulin resistance can be represented as the opposite of insulin sensitivity or as any reduction of insulin sensitivity

Insulin receptor substrate 1 (IRS-1): a signaling protein associated with insulin receptor that acts as a transducer in the insulin-signaling cascade process. Its activation/ phosphorylation leads to an interaction with different second messengers (such as phosphatidylinositol-3-Kinase) involved in various pathways mediating the intracellular action of insulin.

Glucose Tolerance Test (GTT): an experimental procedure allowing to assess the response to an oral or an intravenous load of glucose.

Lipid droplet (LD): intracellular organelle consisting of a core of lipids covered by a monolayer of phospholipids and proteins, among which perilipin (PLIN) proteins that regulate their storage and use [96]. LD store IMTG and bioactive lipid intermediates such as diacylglycerol (DAG) and ceramide [97].

Lipotoxicity: the process by which fatty acids flow into tissues excessively, overwhelm β oxidation machinery and results in subsequent metabolic derangement.

Matsuda Index: An index to evaluate whole body insulin sensitivity from the data obtained by oral GTT.

Perilipin (PLIN): a family of lipid coating proteins that play a vital role in IMTG storage and use.

Protein kinase C (PKC): a family of protein kinase enzymes (details in Box 3)

Very low-density lipoprotein (VLDL): a lipoprotein synthesized in the liver that enable fats and cholesterol to move within the bloodstream.

References

1. Organization, W.H. (2018, February 16) Obesity and overweight fact sheets https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (accessed).

2. Federation, I.D. (2017, December 31) IDF Diabetes Atlas - 8th edition. http://www.diabetesatlas.org/, (accessed).

3. DeFronzo, R.A. and Ferrannini, E. (1991) Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 14 (3), 173-94.

4. Lillioja, S. et al. (1993) Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus: prospective studies of Pima Indians. New England Journal of Medicine 329 (27), 1988-1992.

5. Saltiel, A.R. (2000) Series introduction: the molecular and physiological basis of insulin resistance: emerging implications for metabolic and cardiovascular diseases. J Clin Invest 106 (2), 163-4.

6. Reaven, G.M. (1988) Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 37 (12), 1595-607.

7. Ng, J.M. et al. (2012) PET imaging reveals distinctive roles for different regional adipose tissue depots in systemic glucose metabolism in nonobese humans. Am J Physiol Endocrinol Metab 303 (9), E1134-41.

8. Boersma, G.J. et al. (2018) Altered Glucose Uptake in Muscle, Visceral Adipose Tissue, and Brain Predict Whole-Body Insulin Resistance and may Contribute to the Development of Type 2 Diabetes: A Combined PET/MR Study. Horm Metab Res 50 (8), e10.

9. Petersen, K.F. et al. (2007) The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. Proc Natl Acad Sci U S A 104 (31), 12587-94.

10. DeFronzo, R.A. and Tripathy, D. (2009) Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. Diabetes Care 32 Suppl 2, S157-63.

11. Goodpaster, B.H. and Sparks, L.M. (2017) Metabolic Flexibility in Health and Disease. Cell Metab 25 (5), 1027-1036.

12. Pearson, T. et al. (2016) The Effects of Insulin Resistance on Individual Tissues: An Application of a Mathematical Model of Metabolism in Humans. Bull Math Biol 78 (6), 1189-217.

13. Kelley, D.E. and Mandarino, L.J. (2000) Fuel selection in human skeletal muscle in insulin resistance: a reexamination. Diabetes 49 (5), 677-83.

14. Unger, R.H. (1995) Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. Diabetes 44 (8), 863-70.

15. Unger, R.H. and Zhou, Y.T. (2001) Lipotoxicity of beta-cells in obesity and in other causes of fatty acid spillover. Diabetes 50 Suppl 1 (suppl 1), S118-21.

16. Goodpaster, B.H. et al. (2000) Intramuscular lipid content is increased in obesity and decreased by weight loss. Metabolism 49 (4), 467-472.

17. Krssak, M. et al. (2000) Intramuscular glycogen and intramyocellular lipid utilization during prolonged exercise and recovery in man: a 13C and 1H nuclear magnetic resonance spectroscopy study. J Clin Endocrinol Metab 85 (2), 748-54.

18. Pan, D.A. et al. (1997) Skeletal muscle triglyceride levels are inversely related to insulin action. Diabetes 46 (6), 983-8.

19. Perseghin, G. et al. (1999) Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a 1H-13C nuclear magnetic resonance

531 spectroscopy assessment in offspring of type 2 diabetic parents. Diabetes 48 (8),
532 1600-1606.
533 20. van Loon, L.J. et al. (2004) Intramyocellular lipid content in type 2 diabetes
534 patients compared with overweight sedentary men and highly trained endurance
535 athletes. Am J Physiol Endocrinol Metab 287 (3), E558-65.
536 21. Goodpaster, B.H. et al. (2001) Skeletal muscle lipid content and insulin
537 resistance: evidence for a paradox in endurance-trained athletes. J Clin Endocrinol
538 Metab 86 (12), 5755-61.
539 22. Summers, S.A. (2006) Ceramides in insulin resistance and lipotoxicity. Progress
540 in lipid research 45 (1), 42-72.
541 23. Amati, F. (2012) Revisiting the diacylglycerol-induced insulin resistance
542 hypothesis. Obesity Reviews 13 (S2), 40-50.
543 24. Moro, C. et al. (2008) Determinants of intramyocellular triglyceride turnover:
544 implications for insulin sensitivity. Am J Physiol Endocrinol Metab 294 (2), E203-13.
545 25. Eichmann, T.O. and Lass, A. (2015) DAG tales: the multiple faces of
546 diacylglycerol--stereochemistry, metabolism, and signaling. Cell Mol Life Sci 72 (20),
547 3931-52.
548 26. Eichmann, T.O. et al. (2012) Studies on the substrate and stereo/regioselectivity
549 of adipose triglyceride lipase, hormone-sensitive lipase, and diacylglycerol-O-
550 acyltransferases. J Biol Chem 287 (49), 41446-57.
551 27. Boni, L.T. and Rando, R.R. (1985) The nature of protein kinase C activation by
552 physically defined phospholipid vesicles and diacylglycerols. J Biol Chem 260 (19),
553 10819-25.

554 28. Hannun, Y.A. et al. (1986) Protein kinase C activation in mixed micelles.
555 Mechanistic implications of phospholipid, diacylglycerol, and calcium
556 interdependencies. J Biol Chem 261 (16), 7184-90.
557 29. Prentki, M. and Madiraju, S.R. (2008) Glycerolipid metabolism and signaling in
558 health and disease. Endocr Rev 29 (6), 647-76.
559 30. Itani, S.I. et al. (2002) Lipid-induced insulin resistance in human muscle is
560 associated with changes in diacylglycerol, protein kinase C, and IκB-α. Diabetes 51
561 (7), 2005-2011.
562 31. Itani, S.I. et al. (2000) Involvement of protein kinase C in human skeletal muscle
563 insulin resistance and obesity. Diabetes 49 (8), 1353-8.
564 32. Erion, D.M. and Shulman, G.I. (2010) Diacylglycerol-mediated insulin resistance.
565 Nat Med 16 (4), 400-2.
566 33. Mori, T. et al. (1982) Specificity of the fatty acyl moieties of diacylglycerol for the
567 activation of calcium-activated, phospholipid-dependent protein kinase. J Biochem 91
568 (2), 427-31.
569 34. Ebeling, J.G. et al. (1985) Diacylglycerols mimic phorbol diester induction of
570 leukemic cell differentiation. Proc Natl Acad Sci U S A 82 (3), 815-9.
571 35. Moro, C. et al. (2009) Influence of gender, obesity, and muscle lipase activity on
572 intramyocellular lipids in sedentary individuals. J Clin Endocrinol Metab 94 (9), 3440-
573 7.
574 36. van Hees, A.M. et al. (2011) Skeletal muscle fatty acid handling in insulin
575 resistant men. Obesity (Silver Spring) 19 (7), 1350-9.
576 37. Thrush, A.B. et al. (2009) Skeletal muscle lipogenic protein expression is not
577 different between lean and obese individuals: a potential factor in ceramide

578 accumulation. The Journal of Clinical Endocrinology & Metabolism 94 (12), 5053-
579 5061.
580 38. Coen, P. et al. (2013) Reduced skeletal muscle oxidative capacity and elevated
581 ceramide but not diacylglycerol content in severe obesity. Obesity 21 (11), 2362-
582 2371.
583 39. Liang, H. et al. (2013) Effect of a sustained reduction in plasma free fatty acid
584 concentration on insulin signalling and inflammation in skeletal muscle from human
585 subjects. J Physiol 591 (11), 2897-909.
586 40. Devries, M.C. et al. (2013) Endurance training modulates intramyocellular lipid
587 compartmentalization and morphology in skeletal muscle of lean and obese women.
588 J Clin Endocrinol Metab 98 (12), 4852-62.
589 41. Louche, K. et al. (2013) Endurance exercise training up-regulates lipolytic
590 proteins and reduces triglyceride content in skeletal muscle of obese subjects. J Clin
591 Endocrinol Metab 98 (12), 4863-71.
592 42. Chow, L.S. et al. (2014) Training status diverges muscle diacylglycerol
593 accumulation during free fatty acid elevation. Am J Physiol Endocrinol Metab 307 (1),
594 E124-31.
595 43. Hussey, S.E. et al. (2014) A sustained increase in plasma NEFA upregulates the
596 Toll-like receptor network in human muscle. Diabetologia 57 (3), 582-91.
597 44. de la Maza, M.P. et al. (2015) Skeletal muscle ceramide species in men with
598 abdominal obesity. J Nutr Health Aging 19 (4), 389-96.
599 45. Søgaard, D. et al. (2016) Training does not alter muscle Ceramide and
600 Diacylglycerol in offsprings of type 2 diabetic patients despite improved insulin
601 sensitivity. Journal of diabetes research 2016.

602 46. Goossens, G.H. et al. (2016) Altered skeletal muscle fatty acid handling in
603 subjects with impaired glucose tolerance as compared to impaired fasting glucose.
604 Nutrients 8 (3), 164.
605 47. Shepherd, S.O. et al. (2017) Lipid droplet remodelling and reduced muscle
606 ceramides following sprint interval and moderate-intensity continuous exercise
607 training in obese males. Int J Obes (Lond) 41 (12), 1745-1754.
608 48. Lundsgaard, A.M. et al. (2017) Opposite Regulation of Insulin Sensitivity by
609 Dietary Lipid Versus Carbohydrate Excess. Diabetes 66 (10), 2583-2595.
610 49. Bak, A.M. et al. (2018) Prolonged fasting-induced metabolic signatures in human
611 skeletal muscle of lean and obese men. PLoS One 13 (9), e0200817.
612 50. Bergman, B.C. et al. (2018) Intramuscular triglyceride synthesis: importance in
613 muscle lipid partitioning in humans. Am J Physiol Endocrinol Metab 314 (2), E152-
614 E164.
615 51. Perreault, L. et al. (2018) Intracellular localization of diacylglycerols and
616 sphingolipids influences insulin sensitivity and mitochondrial function in human
617 skeletal muscle. JCI Insight 3 (3).
618 52. Sogaard, D. et al. (2019) The Influence of Age and Cardiorespiratory Fitness on
619 Bioactive Lipids in Muscle. J Gerontol A Biol Sci Med Sci 74 (6), 778-786.
620 53. Sogaard, D. et al. (2019) Muscle-Saturated Bioactive Lipids Are Increased with
621 Aging and Influenced by High-Intensity Interval Training. Int J Mol Sci 20 (5).
622 54. Amati, F. et al. (2011) Skeletal-muscle triglycerides, diacylglycerols, and
623 ceramides in insulin resistance: another paradox in endurance-trained athletes?
624 Diabetes, DB_101221.
625 55. Bergman, B.C. et al. (2012) Localisation and composition of skeletal muscle
626 diacylglycerol predicts insulin resistance in humans. Diabetologia 55 (4), 1140-50.

627 56. Jocken, J.W. et al. (2013) Insulin-mediated suppression of lipolysis in adipose
628 tissue and skeletal muscle of obese type 2 diabetic men and men with normal
629 glucose tolerance. Diabetologia 56 (10), 2255-65.
630 57. Nowotny, B. et al. (2013) Mechanisms underlying the onset of oral lipid-induced
631 skeletal muscle insulin resistance in humans. Diabetes 62 (7), 2240-8.
632 58. Szendroedi, J. et al. (2014) Role of diacylglycerol activation of PKCtheta in lipid-
633 induced muscle insulin resistance in humans. Proc Natl Acad Sci U S A 111 (26),
634 9597-602.
635 59. Tonks, K.T. et al. (2016) Skeletal muscle and plasma lipidomic signatures of
636 insulin resistance and overweight/obesity in humans. Obesity (Silver Spring) 24 (4),
637 908-16.
638 60. Amati, F. (2012) Revisiting the diacylglycerol-induced insulin resistance
639 hypothesis. Obes Rev 13 Suppl 2 (S2), 40-50.
640 61. Dube, J.J. et al. (2007) Leptin, skeletal muscle lipids, and lipid-induced insulin
641 resistance. Am J Physiol Regul Integr Comp Physiol 293 (2), R642-50.
642 62. Preiss, J. et al. (1986) Quantitative measurement of sn-1,2-diacylglycerols
643 present in platelets, hepatocytes, and ras- and sis-transformed normal rat kidney
644 cells. J Biol Chem 261 (19), 8597-600.
645 63. Dube, J.J. et al. (2011) Effects of weight loss and exercise on insulin resistance,
646 and intramyocellular triacylglycerol, diacylglycerol and ceramide. Diabetologia 54 (5),
647 1147-56.
648 64. Bang, H.O. et al. (1980) The composition of the Eskimo food in north western
649 Greenland. Am J Clin Nutr 33 (12), 2657-61.
650 65. Bosma, M. et al. (2012) Perilipin 2 improves insulin sensitivity in skeletal muscle
651 despite elevated intramuscular lipid levels. Diabetes 61 (11), 2679-90.

66. Newsom, S.A. et al. (2015) Lipid mixtures containing a very high proportion of saturated fatty acids only modestly impair insulin signaling in cultured muscle cells. PloS one 10 (3), e0120871.

67. Capel, F. et al. (2016) Oleate dose-dependently regulates palmitate metabolism and insulin signaling in C2C12 myotubes. Biochim Biophys Acta 1861 (12 Pt A), 2000-2010.

68. Miklosz, A. et al. (2017) Challenging of AS160/TBC1D4 Alters Intracellular Lipid milieu in L6 Myotubes Incubated With Palmitate. J Cell Physiol 232 (9), 2373-2386.

69. Jefferson, G.E. et al. (2017) Calcitriol concomitantly enhances insulin sensitivity and alters myocellular lipid partitioning in high fat-treated skeletal muscle cells. J Physiol Biochem 73 (4), 613-621.

70. Pillon, N.J. et al. (2018) Cell-autonomous sphingolipid changes do not underlie fatty acid-induced insulin resistance of GLUT4 translocation or pro-inflammatory signaling in muscle cells. Journal of Lipid Research, jlr. M080788.

71. Timmers, S. et al. (2012) Augmenting muscle diacylglycerol and triacylglycerol content by blocking fatty acid oxidation does not impede insulin sensitivity. Proceedings of the National Academy of Sciences 109 (29), 11711-11716.

72. Franko, A. et al. (2012) Complete failure of insulin-transmitted signaling, but not obesity-induced insulin resistance, impairs respiratory chain function in muscle. Journal of molecular medicine 90 (10), 1145-1160.

73. Bosma, M. et al. (2013) Overexpression of PLIN5 in skeletal muscle promotes oxidative gene expression and intramyocellular lipid content without compromising insulin sensitivity. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids 1831 (4), 844-852.

74. Badin, P.-M. et al. (2013) High-fat diet-mediated lipotoxicity and insulin resistance is related to impaired lipase expression in mouse skeletal muscle. Endocrinology 154 (4), 1444-1453.

75. Turner, N. et al. (2013) Distinct patterns of tissue-specific lipid accumulation during the induction of insulin resistance in mice by high-fat feeding. Diabetologia 56 (7), 1638-48.

76. Bruce, C.R. et al. (2013) The sphingosine-1-phosphate analog FTY720 reduces muscle ceramide content and improves glucose tolerance in high fat-fed male mice. Endocrinology 154 (1), 65-76.

77. Holloway, G.P. et al. (2014) Chronic muscle stimulation improves insulin sensitivity while increasing subcellular lipid droplets and reducing selected diacylglycerol and ceramide species in obese Zucker rats. Diabetologia 57 (4), 832-840.

78. Mason, R.R. et al. (2014) PLIN5 deletion remodels intracellular lipid composition and causes insulin resistance in muscle. Mol Metab 3 (6), 652-63.

79. Selathurai, A. et al. (2015) The CDP-ethanolamine pathway regulates skeletal muscle diacylglycerol content and mitochondrial biogenesis without altering insulin sensitivity. Cell metabolism 21 (5), 718-730.

80. Matravadia, S. et al. (2016) LA and ALA prevent glucose intolerance in obese male rats without reducing reactive lipid content, but cause tissue-specific changes in fatty acid composition. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 310 (7), R619-R630.

81. Serup, A.K. et al. (2016) Partial Disruption of Lipolysis Increases Post Exercise Insulin Sensitivity in Skeletal Muscle Despite Accumulation of DAG. Diabetes 65 (10):2932-42

701 82. Zabielski, P. et al. (2017) Effect of metformin on bioactive lipid metabolism in
702 insulin-resistant muscle. J Endocrinol 233 (3), 329-340.
703 83. Bell, K.S. et al. (2000) Acute reversal of lipid-induced muscle insulin resistance is
704 associated with rapid alteration in PKC-theta localization. Am J Physiol Endocrinol
705 Metab 279 (5), E1196-201.
706 84. Schmitz-Peiffer, C. (2013) The tail wagging the dog–regulation of lipid
707 metabolism by protein kinase C. The FEBS journal 280 (21), 5371-5383.
708 85. Yu, C. et al. (2002) Mechanism by which fatty acids inhibit insulin activation of
709 insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity
710 in muscle. Journal of Biological Chemistry 277 (52), 50230-50236.
711 86. Griffin, M.E. et al. (1999) Free fatty acid-induced insulin resistance is associated
712 with activation of protein kinase C theta and alterations in the insulin signaling
713 cascade. Diabetes 48 (6), 1270-1274.
714 87. MacPherson, R.E. and Peters, S.J. (2015) Piecing together the puzzle of perilipin
715 proteins and skeletal muscle lipolysis. Appl Physiol Nutr Metab 40 (7), 641-51.
716 88. Gemmink, A. et al. (2017) Intramyocellular lipid droplets and insulin sensitivity,
717 the human perspective. Biochim Biophys Acta Mol Cell Biol Lipids 1862 (10 Pt B),
718 1242-1249.
719 89. Mellor, H. and Parker, P.J. (1998) The extended protein kinase C superfamily.
720 Biochem J 332 (Pt 2) (2), 281-92.
721 90. Steinberg, S.F. (2008) Structural basis of protein kinase C isoform function.
722 Physiol Rev 88 (4), 1341-78.
723 91. Newton, A.C. (2010) Protein kinase C: poised to signal. Am J Physiol Endocrinol
724 Metab 298 (3), E395-402.

725 92. Coussens, L. et al. (1987) Alternative splicing increases the diversity of the
726 human protein kinase C family. DNA 6 (5), 389-94.
727 93. Dries, D.R. et al. (2007) A single residue in the C1 domain sensitizes novel
728 protein kinase C isoforms to cellular diacylglycerol production. J Biol Chem 282 (2),
729 826-30.
730 94. Schmitz-Peiffer, C. and Biden, T.J. (2008) Protein kinase C function in muscle,
731 liver, and beta-cells and its therapeutic implications for type 2 diabetes. Diabetes 57
732 (7), 1774-83.
733 95. Rando, R.R. and Young, N. (1984) The stereospecific activation of protein kinase
734 C. Biochem Biophys Res Commun 122 (2), 818-23.
735 96. Shaw, C.S. et al. (2008) Network distribution of mitochondria and lipid droplets in
736 human muscle fibres. Histochem Cell Biol 129 (1), 65-72.
737 97. DiAugustine, R.P. et al. (1973) Hepatic lipid droplets. Isolation, morphology and
738 composition. Biochem J 132 (2), 323-7.

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Table 1: Studies showing no associations between muscle DAG content and IR

First Author	Year	Design	Subjects (N) & Gender	Intervention, *Muscle* & DAG measurement	Results	Ref
Coen	2013	CS	L (8), OB class I (7), II & III (15) women	*Vastus lateralis* HPLC-MS/MS	OB class II&III had greater IR (assessed via HOMA-IR) compared with L and OB class I. Total DAG content as well as DAG moieties were not different among groups. IR was not correlated with DAG. FA oxidation was not related to any lipid species but was related to Mito content. DAG species abundance presented in supplemental table S1.	[38]
Liang	2013	I	L (17), OB (14), OB T2D (12) sedentary women and men	Acipimox 250 mg every 6 h for 8 days. *Vastus lateralis* Radiolabeling DAG kinase - TLC	OB and T2D had greater IR than L (HOMA-IR and HE clamp) . DAG content was similar in all groups at baseline. Acipimox decreased FFA, improved IS in OB and T2D. Total DAG and ceramide content did not change in response to Acipimox.	[39]
Devries	2013	I	L (12), OB (11) sedentary women	E 12-week ET *Vastus lateralis* Radiolabeling DAG kinase - TLC	OB had greater IR than L (HOMA-IR). DAG content was not different between groups at baseline. ET had no effect on total DAG and IR. ET decreased IMCL in the subsarcolemmal region, increased intermyofibrillar IMCL and increased mito content in both subcellular regions.	[40]
Louche	2013	I	OB men (10)	E 8-week ET *Vastus lateralis* Gas-LC	ET improved whole-body aerobic capacity but did not influence glucose tolerance (OGTT) or plasma lipids. ET reduced IMTG, improved FA oxidation and lipases content, but did not change total DAG content.	[41]
Chow	2014	I	L sedentary (13), A (15) women and men	LI *Vastus lateralis* LC-MS	A had higher IS than L. Baseline total TAG, total DAG, and saturated DAG were not different between groups. LI produced similar elevation of FFA and decline of IS (HE clamp) in both groups.LI increased total DAG and DAG containing C18:1-, C18:2-, C18:3- in L but not in A.	[42]
Hussey	2014	I	Sedentary L NGT (12) women and men	LI *Vastus lateralis* LC-MS/MS	LI reduced IS (HE clamp and HOMA IR) and p-IRS-1^{tyr612} without affecting total DAG. LI modified two DAG species in opposite directions: C14:0-C18:1 and C18:0-C20:4 (text and graphs show different directions, see supplemental table S1).	[43]
De la	2015	CS	Healthy	*Anterior abdominal*	Total DAG did not relate with obesity, IR and age.	[44]

Maza			sedentary males (56)	*oblique* LC-MS/MS	DAG species were not different when subjects were stratified by abdominal adiposity. DAG species abundance presented in supplemental table S1.	
Sogaard	2016	I	Sedentary L (16), T2D offspring (19) women and men	E 10-week ET *Vastus lateralis* UPLC-MS	IS (HE clamp) was higher in L compared to offsprings despite similar total DAG or DAG species. IS and total DAG were not related at baseline. IS was improved in both groups after ET without any change in total DAG or DAG species. Changes in IS with intervention were not related with changes in total DAG content. PKCθ or p-PKCθ^{ser676} protein expression were similar in both groups before or after ET. DAG species abundance presented in supplemental table S1.	[45]
Goossens	2016	CS	IFG (12), IGT (14) women and men	*Vastus lateralis* TLC	IGT had lower IS than IFG (HE clamp). IGT had higher TAG and lower DAG content than IFG. IGT increased SM VLDL-TAG extraction and reduced lipid turnover of saturated FA in response to high fat meal compared to IFG.	[46]
Shepherd	2017	I	OB sedentary men (16)	E Randomized 4 weeks of SIT or MICT *Vastus lateralis* Electrospray ionization-MS	IS (Matsuda index) increased by both SIT (11%) and MICT (24%), with no significant difference between groups. IMTG and total DAG did not change with either training protocols. Mito content increased similarly in both groups as well as IMGT in contact with Mito. DAG containing C18:1-C18:2- and DAGC18:0-C18:2 increased similarly in both groups.	[47]
Lundsgaard	2017	I	Healthy men (9)	D Randomized crossover 3 days hypercaloric high unsaturated FA (UNSAT), hypercaloric carbohydrate (CHO), eucaloric diet (CON) *Vastus lateralis* TLC	UNSAT (compared with CON) decreased whole-body IS and insulin-stimulated leg glucose uptake (HE clamp). UNSAT elevated IMTG content (52%) and 1,3 DAG (37%). without changes in insulin signaling cascade (p-AKT^{Thr308}). Decreases in p-HSL^{Ser660} suggested reduced DAG hydrolysis.	[48]
Bak	2018	I	L (9) and OB (9) men	D Randomized crossover 72 h vs 12 h	OB had ~50% lower whole body IS level compared with L after 12h fasting. Prolonged fasting decreased further IS by ~50% in L and ~25% in OB reaching similar absolute levels of glucose infusion rate after 72h fasting. Similar increments in IMTG were observed in both groups in response to 72h fasting.	[49]

				overnight fast *Vastus lateralis* Non-targeted gas chromatography-MS and LC-MS	1,2 DAG and 1,3 DAG were not elevated in OB compared with L and were not modified after 72h fasting in any of the groups. Insulin-stimulated p- Akt^{Ser473} and ^{Thr308} were lower in OB than Lafter 12 h but similar between groups after 72h fasting.	
Bergman	2018	CS	OB (14),T2D (15), A (15) women and men	*Vastus lateralis* HPLC-MS	A were more IS than OB and T2D (IVGTT). Palmitate oxidation was similar at rest and increased in all groups during an acute bout of exercise, with a greater increment in A. IMTG were similar in all groups at rest. During exercise IMTG did not change in OB and T2D, but decreased in A. IMTG synthesis at rest was greater in A. During exercise IMTG synthesis increased in all groups and decreased during recovery. DAG content were similar in all groups at all times. Resting IMTG synthesis was associated with IS and with cytosolic DAG content, but not with whole DAG or membrane DAG content.	[50]
Perreault	2018	CS	Sedentary L (15), OB (15), T2D (12), A (16) women and men	*Vastus lateralis* HPLC-MS	T2D were more IR than OB, which were more IR than L, which were more IR than A (HE clamp). A and T2D had the highest content of total DAG. Only 1,2 DAG were different among groups with higher contents in A and T2D. Sarcolemma DAG were more abundant in A and T2D compared to L. Membrane 1,2 DAG were higher in A, OB and T2D compared to L. Sarcolemma disaturated 1,2-DAG were negatively related with IS if A were removed (not significant in whole cohort). Mito and ER DAG, particularly 1,2 DAG, were more abundant in L and A compared with OB. Mito and ER 1,2 DAG positively associated with IS. Desaturated 1,2 DAG inversely associated with IS. Nuclear DAG were grater in A compared to L and OB, with a positive relationship with IS. Cytosolic DAG were not different among groups and with no relationship with IS. De novo DAG synthesis did not explain DAG repartition and accumulation. PKCε activation was higher in OB and T2D compared with L and A, without significant differences for PKCθ, PKCδ, or PKCβII. Positive relationship between PKCε and sarcolemma 1,2 DAG C16:0-C18:2. There were no significant relationships between 1,2-DAG in any other compartment and PKCε. No significant relationships were found between sarcolemmal 1,2-DAGs and PKCθ, PKCδ, or PKCβII. 1,2 DAG were more abundant (60%) than 1,3 and 2,3 DAG. The more abundant 1,2 DAG in Mito ER were 16:0 18:1 (higher in A), then DiC18:1 (also more in A), 16:0-18:2 (more in A). Same abundances in membrane, nuclear and cytosolic DAG but without significant differences between groups.	[51]

Sogaard	2019	CS	Young untrained (11) and trained (16), aged untrained (18) and trained (15) men	*Vastus lateralis* TLC	Aged had higher IR (HOMA-IR) than young. DAG were not different across trained and untrained states. Only DAG containing C24:0- was higher in young, while C16:1n7 was higher in the aged. None of the DAG FA or total DAG correlated with HOMA-IR.	[52]

Design: CS, cross-sectional; I intervention. *Subjects:* L, lean; OB, obese; T2D, individuals with type 2 diabetes; A, chronically endurance trained/athletes; NGT, normal glucose tolerant; IGT, impaired glucose tolerant; IFG, impaired fasting glucose. *Intervention*: E, Exercise; D, dietary; LI, lipid-infusion; ET, endurance training; SIT, sprint interval training; MICT, moderate intensity continuous training. *DAG measurement/technique:* HPLC, high performance liquid chromatography; MS, mass spectrometry; TLC, thin layer chromatography; LC, liquid chromatography; MS/MS, tandem MS; UPLC, ultra performance Liquid chromatography. *Outcome:* IS, insulin sensitivity/sensitive; IR, insulin resistance/resistant; DAG, diacylglycerol; FA, fatty acid; IMCL, intramyocellular lipids; IMTG, intramuscular triglyceride, LD, lipid droplet; VLDL, very low density lipoprotein; TAG, triacylglycerol; PKC, protein kinase C. *Localization:* Mito, mitochondria. *Outcome measurement:* HOMA-IR, homeostatic model assessment; HE clamp, hyperinsulinemic euglycemic clamp; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test.

Table 2: Studies showing an association between skeletal muscle DAG and IR.

First Author	Year	Design	Subjects (N) & Gender	Intervention, *Muscle* & DAG measurement	Results	Ref
Bergman	2012	CS	OB (6), T2D (6), A (10) males and females (only males for T2D)	*Vastus lateralis* HPLC-MS	OB and T2D had higher IR than A (IVGTT). Total DAG and membrane DAG were higher in OB and T2D than A. Cytosolic DAG were lower in T2D compared to OB and A. Specific membrane DAG species higher in T2D: C18:0-C20:4, Di-C16:0 and Di-C18:0. No group differences in cytosolic species. DAG species abundance presented in supplemental table S1. Total and cytosolic DAG species did not correlate with IS. Only membrane DAG and Di-C18:0 correlated negatively with IS. Cytosolic DAG content was negatively and membrane DAG positively associated to PKCε. Specific species leading these associations were in the cytosol: C18:0-C18:1, Di-C14:0, DiC16:0 and Di-C18:0, and in the membrane: C16:0-C18:1 and C16:1-C18:1. No associations with PKCθ activation. Only saturated membrane DAG were related to IR.	[55]
Jocken	2013	CS	NGT (11) and T2D (9) men	*Vastus lateralis* TLC	T2D had higher IR than NGT (HOMA-IR and HE clamp). Total and membrane DAG were higher in T2D. Specific membrane DAG species higher in T2D contained: C16:0-, C17:0-, C18.0-, C22:0- and trans C18:1-. Cytosolic DAG were not different between groups. Saturated membrane DAG were inversely associated with IS and positively associated with PKCδ activation.	[56]
Nowotny	2013	I	L IS (16) men and women	Acute IR induction, randomized crossover 4 conditions: LI, oral fat, LPS and control *Vastus lateralis* LC-MS/MS	LI, oral fat and LPS reduced IS (HE clamp). LI and oral fat increased PKCθ activation. Membrane Di-C18:2 DAG was increased after LI but not oral fat or LPS. Overall membrane DAG and membrane Di-C18:2 DAG were positively correlated with PKCθ activation after oral fat but not LI or LPS. LPS raised IR through the stimulation of inflammatory pathways.	[57]
Szendroedi	2014	CS	Sedentary L NGT (36), OB IR (10), OB T2D (	LI in L *Vastus lateralis* LC-MS/MS	OB and T2D were similarly more IR than L (HE clamp) and had higher contents of total and cytosolic DAG than L. Membrane DAG were higher in T2D than OB and L. Specific cytosolic DAG higher in OB and T2D : C16:0-C18:2, Di-C18:2, C18:1-	[58]

			10) women and men		C18:2, C18:0-C16:0, Di-C16:0, C18:1-C18:0, C18:2-C18:0, C18:0-C20:4, C16:0-C20:4, C18:1-C16:0. Specific membrane DAG higher in OB and T2D were Di-C18:0 and the same as above but not Di-C16:0 and C18:1-C16:0. Total cytosolic DAG correlated negatively with IS as well as cytosolic species containing C18:0-C18:2 and C16:0-C18:2 and membrane species containing C18:0-C20:4, C18:2-C18:0, C18:1-C18:2, Di-C18:2, C16:0-C18:2. Other membrane DAG containing C20:4- and C20:5- correlated positively with IS. Activation of PKCθ was higher in OB and T2D than L, without differences in PKCδ and PKCε. PKCθ activation correlated negatively with IS and positively with total cytosolic and membrane DAG, and with the following species at both locations C16:0-C20:4, C16:0-C18:2, C18:0-C20:4, C18:1-C18:2, C18:0-C18:2 and Di-C18:2. In L, acute induction of IR through 4 hours of LI increased total DAG, cytosolic DAG and membrane DAG. Specific cytosolic species increased: C16:0-C18:2, Di-C18:2, C16:0-C20:4. Membrane species that increased are the same than cytosolic and C18:0-C18:2, C18:1-C18:2. LI activated PKCθ, but not PKCδ and PKCε, and increased p-IRS1^{ser1101}.	
Tonks	2016	CS	Sedentary L (23), overweight /OB (14) or OB IR (14) women and men	*Vastus lateralis* LC-electrospray ionization MS	Di-C18:2 DAG was the only DAG different between groups, with lower content in OB IR compared to L. DAG species abundance are presented in supplemental table S1.	[59]

Design: CS, cross-sectional; I intervention. *Subjects:* A, chronically endurance trained/athletes; L, lean; NGT, normal glucose tolerant; OB, obese; T2D, individuals with type 2 diabetes. *Intervention*: LI, lipid-infusion; LPS, intravenous endotoxin. *DAG measurement/technique:* HPLC, high performance liquid chromatography; MS, mass spectrometry; TLC, thin layer chromatography; LC, liquid chromatography; MS/MS, tandem MS; UPLC, ultra performance Liquid chromatography. *Outcome:* DAG, diacylglycerol; FA, fatty acid; IR, insulin resistance/resistant; IS, insulin sensitivity/sensitive; PKC, protein kinase C. *Localization*: ER, endoplasmic reticulum; Mito, mitochondria. *Outcome measurement:* HOMA-IR, homeostatic model assessment; HE clamp, hyperinsulinemic euglycemic clamp; IVGTT, intravenous glucose tolerance test.

Table 3: Mechanistic studies (in vitro) showing associations and dissociations between muscle DAG content and IR

First Author	Year	In vitro model	Experiments	Results	Ref
Bosma	2012	C2C12	PLIN2 OE and KD, Incubation with/without C16:0 or C18:1	PLIN2 KD incubated with C16:0 lowered IMTG, increased C16:0- DAG, without compromising in insulin signaling PLIN 2 OE incubated with C16:0 increased IMTG, increased C16:0-DAG and improved IS thus protecting against C16:0-induced impairments in insulin-stimulated glucose uptake compared to control cells.	[65]
Newsom	2015	C2C12, Human primary cells	Incubation with different concentrations and proportions of FA mixtures	C2C12: Incubation with 100% C16:0 increased DAG content in a dose-dependent manner and impaired insulin signaling (decreased p-Akt^{Thr308}/Akt).The fatty acid composition of DAG resembled the FA provided in the incubation media. Myotubes: Incubation 100% C16:0 increased DAG concentration without significant impairments of insulin signaling.	[66]
Capel	2016	C2C12	Incubation with C16:0 with/without different doses of C18:1	C16:0 incorporation into DAG was reduced by concomitant C18:1. C18:1 co-incubation decreased the impact of C16:0 on the insulin signaling cascade and PKCθ phosphorylation in a dose-specific manner.	[67]
Miklosz	2017	L6	Incubation with/without C16:0	Incubation with C16:0 increased total DAG, the incorporation into DAG of C16:0, C16:1, C18:0, C18:2, C18:3, C20:4, C24:0 and C20:5, and decreased insulin signaling (p-Akt/Akt ratio).	[68]
Jefferson	2017	C2C12	Incubation with/without calcitriol	Calcitriol increased insulin-stimulated p-Akt, increased total DAG, and increased specifically Di-C18:0, C18:1-C20:0, C18:0-C20.4, Di-C18:1, C16:0-C18:1, Di-C14:0, Di-C16:0 and C18:0-C18:2.	[69]
Pillon	2018	L6	Incubation with C16:0 or C16:1	Incubation with C16:0, but not C16:1, impaired insulin signaling and IS without differences in total DAG. DAG containing 16:1 were positively associated with IS.	[70]

In vitro models*:* C2C12, specific cell like from mouse myoblasts (RRID:CVCL_0188); L6, cell line L6 from rat myoblasts (RRID:CVCL_0385). *Experiments:* KD, knock down; OE, overexpression; PLIN, perilipin. *Outcomes:* DAG, diacylglycerol; FA, fatty acid; IMTG, intramyocellular triglyceride; IS, insulin sensitivity; PKC, protein kinase C; p-, phosphorylated; Akt, protein kinase B.

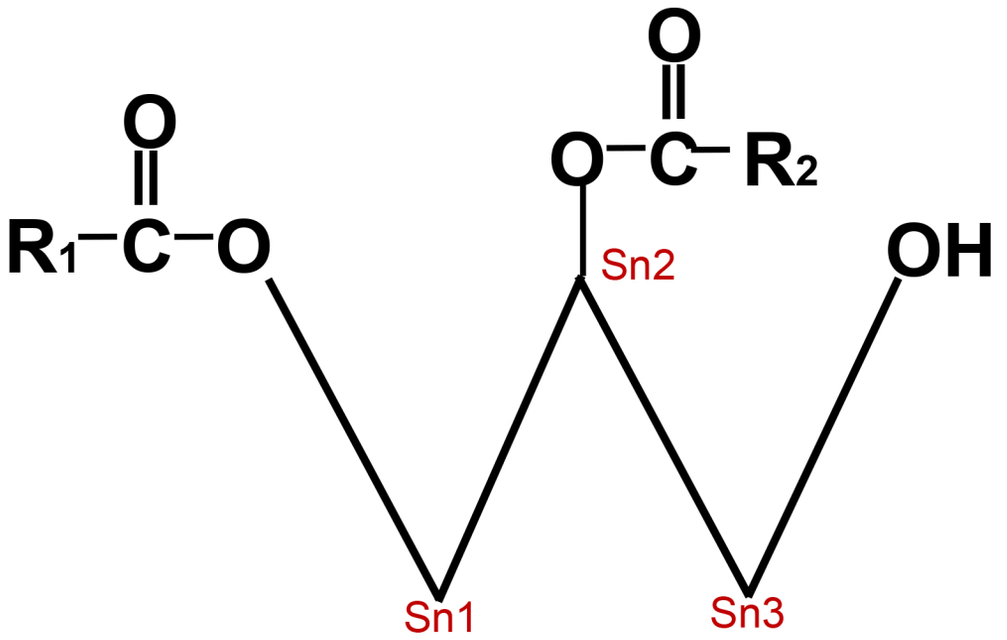
Table 4: Mechanistic studies (Animal models) showing associations and dissociations between muscle DAG content and IR

First Author	Year	Animal model	Diet and/or intervention	Results	Ref
Timmers	2012	14 weeks old male C57BL/6 mice	HFD for 14 days. Etomoxir injections.	Etomoxir increased IMCL and DAG content without increasing PKCθ. Glucose tolerance tests, insulin signaling and insulin-stimulated GLUT4 translocation improved despite the accumulation of DAG response to Etomoxir	[71]
Franko	2012	5-7 months old male mice either: 1) Ob/ob normoglycemic 2) HFD C57BL/6 3) STZ C57BL/6 4) MIRKO	For 2) HFD: 6 months duration starting at 3 months of age For 3) STZ injected at 3 months of age	HFD mice were IR and had higher levels of 1,2 and 1,3 DAG. STZ mice were IR but did not accumulate neither 1,2 nor 1,3 DAG.	[72]
Bosma	2012	7 weeks old male Wistar rats	Low-fat (10% energy from fat) or HFD for 3 weeks. In vivo muscle specific PLIN2 OE (ectroporation) and HE clamp.	PLIN2 OE increased IMTG and improved IS in HFD conditions, without affecting DAG content.	[65]
Bosma	2013	8 weeks old male Wistar rats	HFD. In vivo muscle specific PLIN5 OE and HE clamp.	PLIN5 OE increased IMTG without affecting IS and DAG content.	[73]
Badin	2013	5 weeks old male C3H mice and female HSL null mice	HFD or chow for 4 weeks. HSL KO mice received normal chow for 7 weeks.	C3H mice: HFD increased total DAG content, PKC membrane translocation, impaired insulin signaling and induced IR. HFD mice had higher membrane/cytosol ratio of PKCθ and PKCε. HSL KO mice: higher DAG content, higher C16:0 incorporation into DAG, and impaired insulin signaling.	[74]

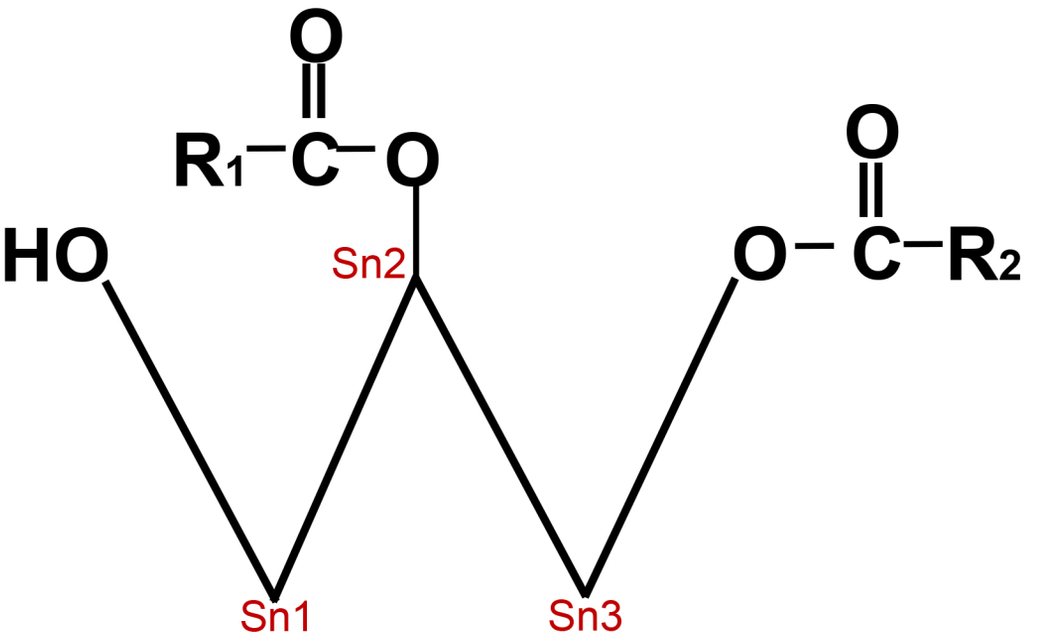
Turner	2013	8-12 weeks old male C57BL/6 mice	HFD vs control diet, from 3 days to 16 weeks. GTT and HE clamp.	Total DAG content was increased at the time that SM IR developed (3 weeks of HFD). At 3 weeks of HFD, the following DAG species were increased compared to control diet: C16:0-C18:1, C16:0-C18:2, C16:1-C18:1, Di-C18:1, C181-C18:2. At 16 weeks of HFD, IR was similar to 3 weeks but DAG content were differentially elevated compared to control diet: C16:0-C18:1, C18:0-C18:1, C18:1-C18:2, C16:0-C20.4, C18:0-C20.4, C16:0-C22.6.	[75]
Bruce	2013	8 weeks old male C57BL/6 mice	HFD vs control diet for 12 weeks with/without FTY720 (5 mg/kg) daily for the last 6 weeks.	In addition of preventing SM Ceramide increases with HFD, FTY720, a sphingosine-1-phosphate analog, prevented increases in total DAG and the following species: C16:0-C18:1, C16:0-C18:2, C18:0-C18:1, Di-C18:1, C18:1-C18:2, C16:0-C20:4, C18:0-C20:4 and C18:1-C20.4. FTY720 improved glucose homeostasis with reduced plasma insulin, improved body glucose tolerance, increased insulin-stimulated glucose uptake, and AKT phosphorylation.	[76]
Holloway	2014	L and OB female Zucker rats (age unavailable)	Chronic contraction*:* *In vivo* unilateral electrical stimulation 6 h/day for 6 days, vs. sham.	At baseline, OB had lower insulin stimulated glucose uptake, higher IMTG and higher total DAG in both red and white muscle. The content of DAG moieties depended on the type of muscle. In red muscle OB had higher DAG containing C14:0-, C16:0, C16:1-, C18:0-and C22:0, but lower C18:n3- and C20:4n6-. In white muscle, OB had higher almost all the DAG measured (12 out of 14). Only DAG containing C20:4n6- and C20-:5n3 were similar in L and OB. Contraction increased insulin stimulated glucose uptake in all muscles both OB and L. IMTG increased in all muscles with +127% in L white, +57% in OB white, +74% in L red and +32% in O red. Neither total nor specific DAG were modified in L muscles or OB white muscle compared to their non stimulated control. In OB red muscle, contraction decreased total DAG by -17%, increased C16:1- (+54%) and decreased C20:0- DAG (-43%).	[77]
Mason	2014	16 weeks old PLIN5 KO male mice vs control	Chow	PLIN5 KO mice are more IR but have similar total DAG content to controls.	[78]
Selathurai	2015	18 weeks old male muscle specific ECT KO mice vs control	Chow	ECT KO mice: 200% accumulation of total DAG, membrane DAG, and specific species including C14:0-C16:0, C14:0-C18:1, Di-C16:0, C16:0-C18:2, C16:1-C18:1, C16:0-C18:0, Di-C18:2, C18:1-C18:2, C18:0-C18:2, Di-C18:1, C18:0-C18.2, C18:0-C20.4. No changes in IS or oxidative capacity.	[79]

Matravadia	2015	6 weeks old male OB Zucker rats	12 weeks of diet containing supplements in C18:2 (LA), C18:3 (ALA) vs control.	LA and ALA did not change total DAG but decreased DAG containing C14:0- and C18:0-. LA increased DAG containing C18:2- and decreased C22:6-. ALA decreased DAG containing C20:4- and increased C18:3-. ALA and LA prevented the elevation of fasting blood glucose and ALA prevented glucose intolerance. Insulin signaling (IRS1) was decreased similarly in all conditions.	[80]
Serup	2016	16-25 weeks old female HSL KO mice vs control	Acute endurance exercise (running)	After exercise: HSL KO mice had higher IS and higher 1,3 DAG compared to controls.	[81]
Zabielski	2017	6 weeks old Male Wistar rats	8 weeks control diet, HFD or HFD with Metformin (Met).	Met prevented HFD induced IR and deteriorations in insulin signaling. HFD triggered increases of total DAG, C16:0-C18:2, Di-C18:0, C18:0-C18:2, Di-C18:2, C18:1-C18:2 and decreased C16:0-C18:0, C16:0-C18:1 and Di-C18:1. Met decreased total DAG and Di-C16:0, increased Di-C18-2 compared to control. Met decreased Di-C16:0, C16:0-C18:0, C16:0-C18:2, C18:0-C18:1, C18:0-C18:2, C18:1-C18:2 and Di-C18:2 compared to HFD alone.	[82]

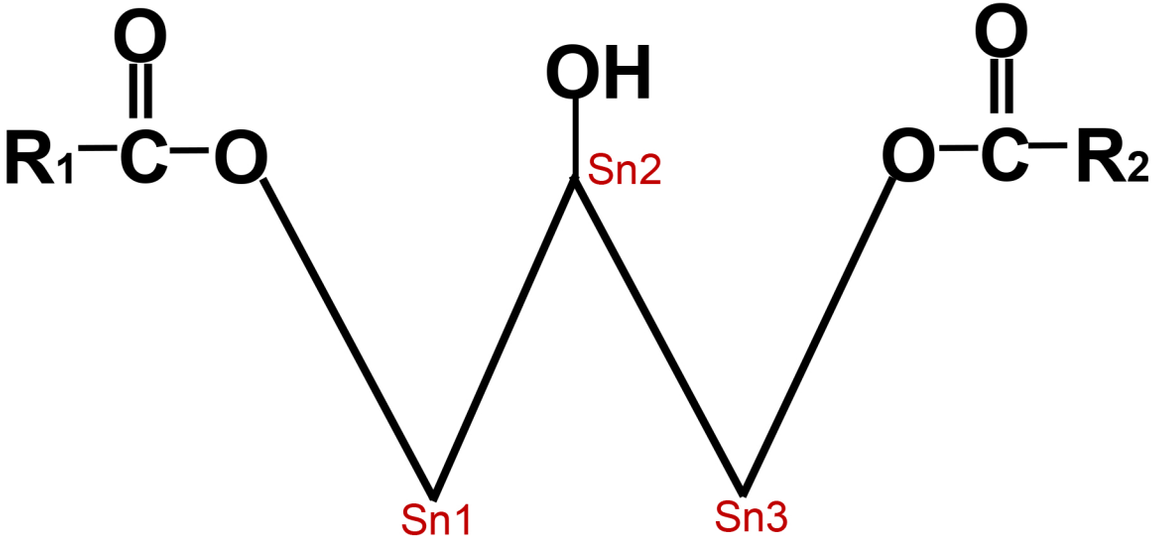
Model: KO: Knock Out; L, Lean; MIRKO muscle-specific insulin receptor knockout mice; Ob/Ob: Obese mice; OB: Obese; PLIN: Perilipin; STZ: Streptozotocin; ECT, phosphoethanolamine cytidylyltransferase; HSL: Hormone Sensitive Lipase; *Intervention*: ALA, Alpha Linolenic Acid; GTT, Glucose Tolerance Test; HE, Hyperinsulinemic Euglycemic Clamp; LA, Linoleic Acid; OE, Overexpression; HFD: High Fat Diet; *Outcomes:* AKT, protein kinase B; GLUT4, glucose transporter type 4; IMCL, intramyocellular lipids; TAG, triacylglycerol. DAG, diacylglycerol; IS, insulin sensitivity; IR, insulin resistance; PKC, protein kinase C; SM, skeletal muscle



1,2-DAG



2,3-DAG



1,3-DAG