J Mol Med (2015) 93:831–843
DOI 10.1007/s00109-015-1310-2

JMolMed

REVIEW

Lipid-mediated muscle insulin resistance: different fat, different pathways?

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Received: 2 March 2015 / Revised: 27 May 2015 / Accepted: 12 June 2015 / Published online: 25 June 2015
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Abstract Increased dietary fat intake and lipolysis result in excessive lipid availability, which relates to impaired insulin sensitivity. Over the last years, several mechanisms possibly underlying lipid-mediated insulin resistance evolved. Lipid intermediates such as diacylglycerols (DAG) associate with changes in insulin sensitivity in many models. DAG activate novel protein kinase C (PKC) isoforms followed by inhibitory serine phosphorylation of insulin receptor substrate 1 (IRS1). Activation of Toll-like receptor 4 (TLR4) raises another lipid class, ceramides (CER), which induce pro-inflammatory pathways and lead to inhibition of Akt phosphorylation. Inhibition of glucosylceramide and ganglioside synthesis results in improved insulin sensitivity and increased activatory tyrosine phosphorylation of IRS1 in the muscle. Incomplete fat oxidation can increase acylcarnitines (ACC), which in turn stimulate pro-inflammatory pathways. This review analyzed the effects of lipid metabolites on insulin action in skeletal muscle of humans and rodents. Despite the evidence for the association of both DAG and CER with insulin resistance, its causal relevance may differ depending on the subcellular localization and the tested cohorts, e.g., athletes. Nevertheless, recent data indicate that individual lipid species and their degree of fatty acid saturation, particularly membrane and cytosolic C18:2 DAG, specifically activate PKCθ and induce both acute lipid-induced and chronic insulin resistance in humans.

Keywords Free fatty acids · Diacylglycerols · Sphingomyelines · Acylcarnitines · Insulin sensitivity

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Introduction

Obesity predisposes for type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD), cardiovascular mortality and cancer [1–3]. All these disorders tightly relate to impaired insulin action, i.e., insulin resistance (IR). Obesity is characterized by excessive accumulation of triacylglycerols (TAG) primarily in adipose tissue [4]. When storage capacity is exceeded, lipids are released into the circulation as fatty acids (FA) and ectopically stored in liver, skeletal muscle, pancreas, and heart. Accumulation of intramyocellular lipids (IMCL) and hepatocellular lipids is generally higher in obesity and T2DM [5, 6] and correlates with IR [7, 8]. Unlike TAG, lipid species such as diacylglycerols (DAG), ceramides (CER), and acylcarnitines (ACC) have been linked to development of IR in different tissues [9]. However, their mechanisms of action and causative role for IR have remained unclear.

Skeletal muscle accounts for approximately 80–90 % of insulin-stimulated glucose disposal in the postprandial state [10]. Thus, we focus here on studies exploring lipid-induced IR in muscle, while the role of other tissues is beyond the scope of this review. We review human and animal studies addressing the effects of lipids, lipid metabolites and their composition on insulin sensitivity, which were accessible in PubMed (2000–2015). The search terms comprised all possible combinations, abbreviations and synonyms of "saturated unsaturated fatty acids insulin resistance", "diacylglycerol

ceramides insulin resistance", "acylcarnitines insulin resistance", "TLR4 inflammation ceramides insulin resistance", "fatty acid metabolism" "lipotoxicity lipid intermediates", and "lipids insulin resistance". Additional references were retrieved from reviewing the references cited in selected articles and the authors' knowledge.

Lipid metabolism and interaction with insulin signaling

Dietary and hepatic TAG are transported by lipoproteins as chylomicrons or VLDL. Membrane-bound lipoprotein lipase releases FA and facilitates their entry into the cell by transport proteins and partially by diffusion [11, 12] (Fig. 1). Within cells, cytoplasmic fatty acid binding protein (FABPc) binds and activates free FA to fatty acyl-CoA (FA-CoA). FA-CoA enter different metabolic pathways, depending on cellular energy demand, FA chain length, and saturation [12, 13]. Transport of long-chain (LC) FA-CoA (13–21 carbons) into the mitochondrial matrix for β-oxidation and tricarboxylic acid (TCA) cycle is regulated by the carnitine shuttle [14]. Short-chain (SC) and medium-chain (MC) FA-CoA (<6 and 6–12 carbons) bypass the carnitine shuttle and serve as ready-to-go energy source [13]. During low energy demand, LCFA-CoA are stored as TAG in droplets or channeled into glycerolipid biosynthesis, while only palmitoyl-CoA serves for sphingolipid synthesis [13, 15]. Overall, intramyocellular lipid metabolism results from a complex interplay between several tightly regulated processes such as lipogenesis, lipolysis, lipid droplets trafficking, and organelle interaction (reviewed in [9, 16]).

The DAG-PKC pathway

DAG consist of two FA bound to glycerol and the most abundant isoform, 1,2-DAG, serves as component of membranes as well as second messenger for signal transduction [17]. Sources of 1,2-DAG are (i) de novo synthesis including esterification of two LCFA-CoA to glycerol-3-phosphate, (ii) cleavage of glycerophospholipids and phosphatidylinositols by phospholipases C and D [18], or (iii) breakdown of TAG (Fig. 1). During de novo synthesis, esterification at the first position of DAG occurs in different subcellular compartments. Saturated FA (SFA) are processed in mitochondria, peroxisomes, and endoplasmic reticulum (ER), while unsaturated FA (UFA) in ER [19]. Hydrolysis of phospholipids yields DAG with different FA composition [18]. Phosphatidylinositol-derived DAG have stearic acid at position 1 and arachidonic acid at position 2, while phosphatidylcholine-derived DAG have mainly SFA and monounsaturated FA (MUFA) at position 2 [18]. Chain length and saturation of FA moieties give rise to many 1,2-DAG species, affecting their physicochemical properties, rates of membrane fusion as well as interactions with membrane-associated proteins [20].

DAG interact and activate proteins containing at least one conserved 1 (C1) domain in their sequence. DAG composition determines the specificity and affinity to C1 domains and allocation of proteins to different intracellular DAG pools, i.e., plasma membrane or lipid droplet (LD) [18]. Novel PKC (nPKC) isoforms PKCθ, PKCδ, PKCβ, and PKCε, are particularly activated by DAG containing at least one UFA and specifically by *n*−3 and *n*−6 polyunsaturated FA [21] with different potencies [22]. Activation of PKCθ induces inhibitory phosphorylation of muscle insulin receptor substrate (IRS) at several residues, but mainly pSer1101 and pSer307 [23, 24] have been linked to decreased phosphatidylinositol 3-kinase (PI3K) and Akt activity and thereby IR [23, 24] (Fig. 2). Other isoforms, such as PKCδ and PKCβ have also been linked to IR in skeletal muscle [25]. In rat liver, PKCδ increased after short-term lipid infusion [26] and PKCε after 3 days on high-fat diet (HFD) [27].

The TLR4-CER pathway

CER are sphingosines covalently bound to one FA and serve as membrane components or second messengers to various cellular stress stimuli [28, 29]. In mammals, more than 200 distinct CER can arise from (i) de novo synthesis by condensation of palmitoyl-CoA and serine via serine palmitoyl transferase and dihydroceramide desaturase (Fig. 1); (ii) sphingomyelin hydrolysis; and (iii) degradation of high-order sphingolipids via the Salvage pathway [29–31].

Increased FA supply and virtually all stress stimuli can raise intracellular CER [28]. Binding of SFA to the Toll-like receptor 4 (TLR4), potentially mediated by fetuin A [32], stimulates de novo synthesis of CER [33] (Fig. 2). In parallel, pro-inflammatory pathways related to IR are activated, including IκB kinase (IKK), mitogen activated protein kinase (MAPK), c-Jun N-terminal kinase (JNK), and cytokine expression [34, 35]. CER accumulation decreases Akt activity and insulin sensitivity independently of IRS1/2 and PI3K [33, 34]. This may result from activation of protein phosphatase 2A, followed by inhibitory Akt dephosphorylation [29].

ACC and incomplete fat oxidation

ACC are formed when LCFA-CoA enter mitochondria for β-oxidation and TCA cycle by the carnitine shuttle. Levels of ACC may vary depending on the metabolic conditions, but can accumulate during decreased β-oxidation or when rates of β-oxidation exceed rates of TCA cycle [14]. Abnormal FA oxidation can lead to impaired mitochondrial function, including the inability to switch to carbohydrate oxidation, depletion of TCA intermediates, and accumulation of ACC, thus contributing to IR [36–38] (Fig. 2). Nevertheless, evidence for a

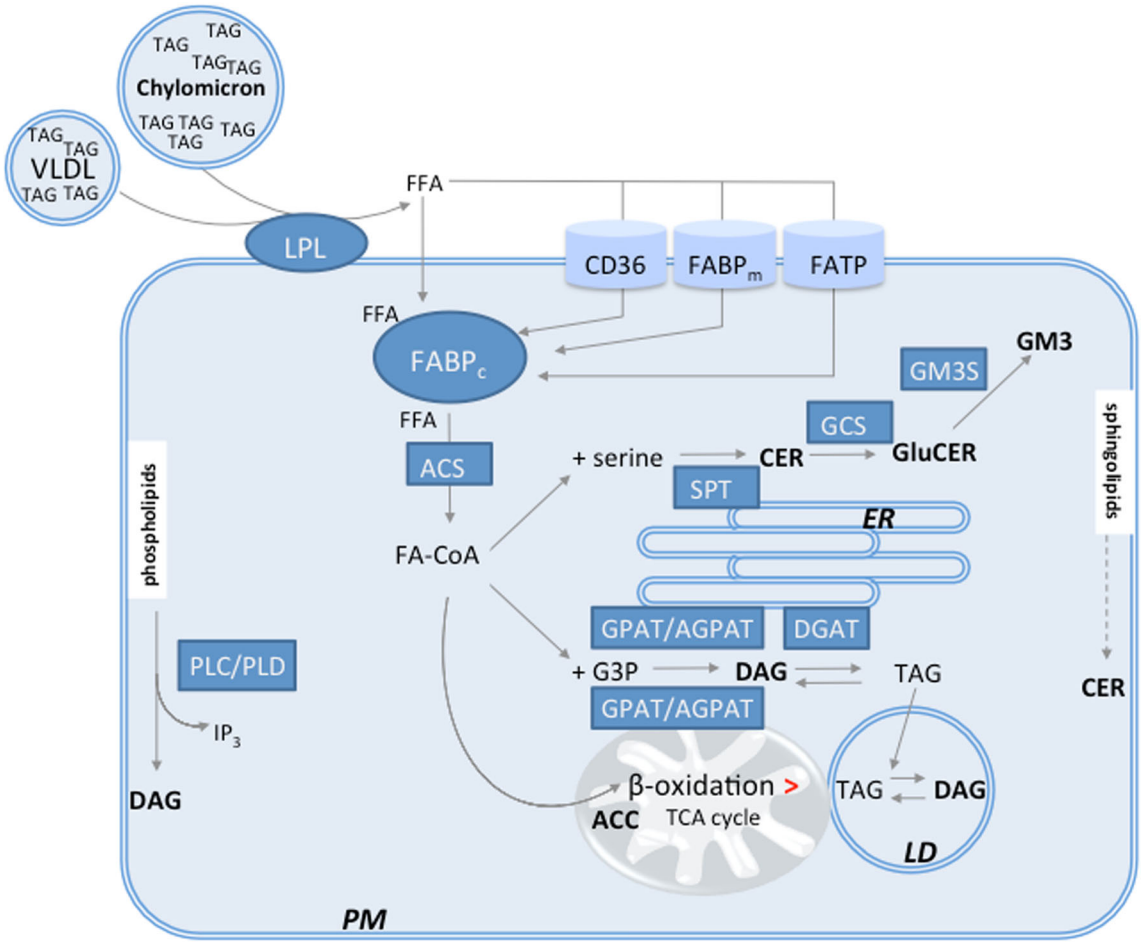

Fig. 1 Metabolic pathways leading to the production of lipid intermediates. After the hydrolysis of circulating triacylglycerols (*TAG*) by membrane-bound lipoprotein lipase (*LPL*), free fatty acids (*FFA*) enter the cell by transport proteins or partially by diffusion. Cytoplasmic fatty acid binding protein (*FABPc*) activates FFA to fatty acyl-CoA (*FA-CoA*), which are redirected to different metabolic pathways: (i) de novo synthesis of ceramides (*CER*) by condensation of palmitoyl-CoA and serine via serine palmitoyl transferase (*SPT*); (ii) de novo synthesis of diacylglycerols (*DAG*) including esterification of two FA-CoA to glycerol-3-phosphate (*G3P*) by glycerol-3-phosphate acyltransferase (*GPAT*) and 1-acylglycerol-3-phosphate acyltransferase (*AGPAT*) and/or (iii) mitochondrial β-oxidation. Decreased or incomplete β-oxidation, i.e., when rates of β-oxidation exceed the rates of tricarboxylic acid (*TCA*) cycle, results in accumulation of acylcarnitines (*ACC*). Glycolysation of CER by glucosylceramide synthase (*GluCER*) yields glucosylceramides (*GluCER*), which can be further converted to ganglioside monosialo 3 (*GM3*) by GM3 synthase (*GM3S*). TAG are produced after the addition of FA-CoA to DAG by diacylglycerol acyltransferase (*DGAT*). Another sources of DAG are hydrolysis of TAG and cleavage of phospholipids by phospholipases C and D (*PLC/PLD*). Finally, CER can be also produced by degradation of sphingolipids. Abbreviations: *ACS* Acyl-CoA synthetase, *CD36* cluster of differentiation 36, *ER* endoplasmic reticulum, *FABPm* membrane fatty acid binding protein, *FATP* fatty acid transport protein, *IP3* inositol triphosphate, *LD* lipid droplet, *PM* plasma membrane, *VLDL* (very low density lipoproteins)

direct interaction between ACC and insulin signaling is missing. ACC species could also induce IR indirectly via NFκB signaling and cytokine secretion as shown in mouse leukemic macrophages and human epithelial cells [39]. The increased pro-inflammatory response can subsequently induce IR in various tissues, including muscle [40].

Other lipids and insulin resistance

Glucosylceramides These sphingolipids are generated via glycosylation of CER by glucosylceramide synthase and can be further transformed into gangliosides [41] (Fig. 1). They are ligands and modulators of receptor activity. The synthesis of ganglioside monosialo 3 (GM3) is induced by TNF-α and paralleled by decreased tyrosine phosphorylation of the insulin receptor and IRS1 (IRS1-tyrPx), resulting in impaired insulin-stimulated glucose uptake in 3T3-L1 adipocytes [42]. However, in vitro studies suggest that GM3 does not induce IR in myocytes [43]. Depending on its acyl chain composition, glucosylceramides (GluCER) can stimulate innate immune cells [44].

Hydroxy fatty acids These lipid intermediates arise as products of lipid oxidation [45]. Patients with diabetes and IR show increased concentrations of hydroxy fatty acids (HFA) in serum and feces [46, 47]. Recently, a new class of HFA,

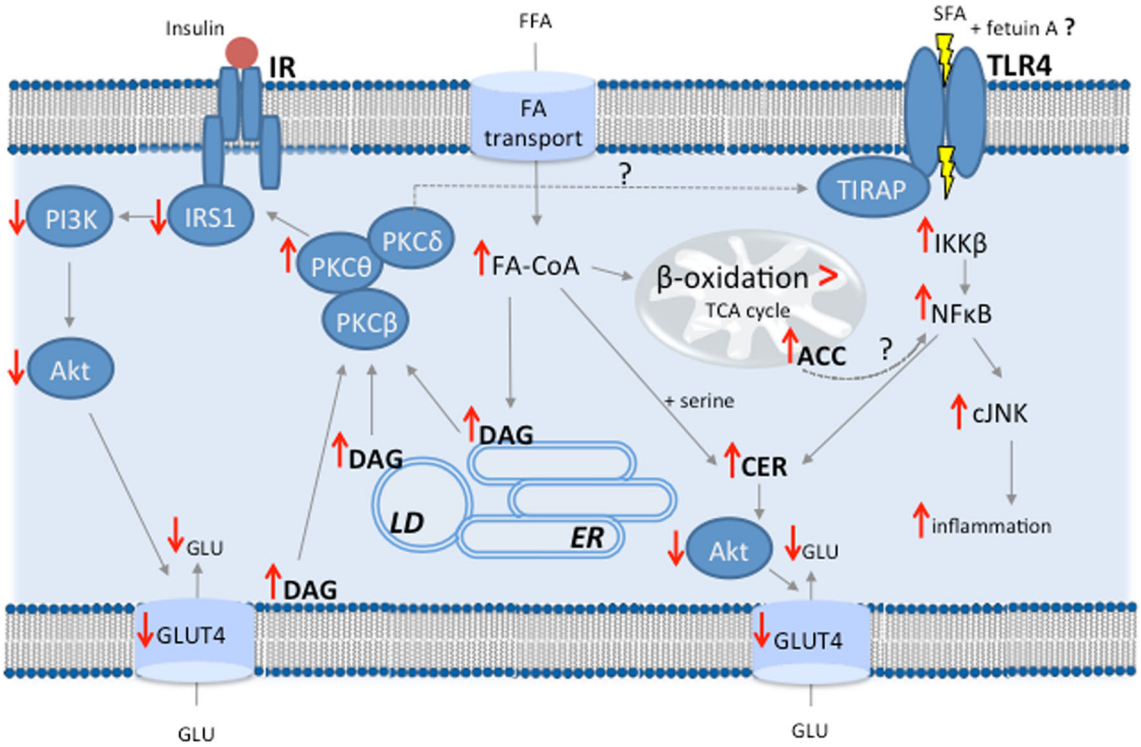

Fig. 2 Interaction of lipid intermediates with insulin signaling and inflammatory pathways in the muscle. Increased diacylglycerols (*DAG*) interact and activate novel isoforms of protein kinase C (*PKC*), such as PKCθ, PKCδ, and PKCβ. Activation of PKCθ in muscle induces inhibitory phosphorylation of insulin receptor substrate 1 (*IRS1*) with subsequent decrease in phosphatidylinositol 3-kinase (*PI3K*), Akt and glucose transporter 4 (*GLUT4*) activities, overall leading to decreased glucose (*GLU*) uptake. Ceramides (*CER*) impair glucose uptake by decreasing the activity of Akt. Acylcarnitines potentially induce the activity of nuclear factor kappa-light-chain-enhancer of activated B-cells (*NFκB*) and inflammatory response. Saturated fatty acids (*SFA*) bind to toll-like receptor 4 (*TLR4*), potentially by fetuin A, inducing inflammatory response and increased CER synthesis. *Abbreviations*: *cJNK* c-Jun N-terminal kinase, *ER* endoplasmic reticulum, *FA* fatty acids, *FFA* free fatty acids, *IR* insulin receptor, *IKKβ* IκB kinase, *LD* lipid droplet, *SFA* saturated fatty acids, *TCA* tricarboxylic acid

palmitic acid hydroxy-stearic acids (PAHSA) have been positively linked to insulin sensitivity in adipose tissue of humans and mice [48] and thus could have metabolically beneficial effects, similar to *n*−3 FA and endogenous palmitoleate [49, 50]. However, the relevance in human muscle may be minor due to low muscular concentrations of PASHA and their downstream mediators [48, 51].

Studies on lipid-mediated insulin resistance

These studies employed experimental alteration of circulating lipids (interventional studies, Tables 1 and 2) or compared groups with different degrees of IR (cross-sectional studies, Table 3) in human and rodent muscles.

Interventional studies

To study early mechanisms underlying lipid-induced IR, protocols of short-term lipid infusion or HFD were developed. At least 3–4 h of lipid infusion are needed to detect a decrease in whole-body insulin-stimulated glucose disposal, due to a sequence of events starting with a relevant raise in circulating insulin (~0.5 h) and FA (1–1.5 h), followed by the intramyocellular accumulation of lipid metabolites (~2.5 h) [60, 75, 76]. Fat emulsions Intralipid 20 % (53 % *n*−6 PUFA, 24 % UFA, 16 % SFA) and Liposyn II (66 % *n*−6 PUFA, 18 % UFA, 12 % SFA) [77, 78] are commonly used and consistently induce IR [79, 80]. On the other hand, a wide range of HFD, differing in energy and fat content and FA composition are employed, leading to variable effects on insulin sensitivity. Most studies assess insulin sensitivity in vivo with hyperinsulinemic-euglycemic clamps (HEC) and/or ex vivo from muscle biopsies at different time points.

Rodents Liposyn II or Intralipid 12 % infusion in rats resulted in IR at 5 h [23, 53], but only Liposyn II infusion was accompanied by intramyocellular DAG accumulation, increased PKCθ activity, decreased IRS1-tyrPx and associated PI3K activity [23]. In contrast, Intralipid 20 % increased Akt activity and IRS1-tyrPx [53]. Uniformly, CER pools were unaffected (Table 1).

Another study directly compared lipid emulsions differing in FA saturation in rats. Infusions of lard oil (rich in SFA) or soybean oil (rich in UFA) induced IR, decreased muscle Akt and increased DAG [52]. However, only lard oil raised CER,

Table 1 Interventional studies in rodents: lipid-mediated insulin resistance (IR) in skeletal muscle

Study group	Intervention	Basal characteristics	Insulin sensitivity (method)	Insulin signaling	Intramyocellular lipids				Ref
					TAG	DAG	CER	ACC	
Wistar rats	iv Liposyn II 20 % (UFA[a]) vs saline for 5 h	–	↓ (HEC)	↑ PKCθ (5 h) ↓ IRS1tyrPx (5 h) ↓ PI3K (5 h)	↔	↑	↔	n.a.	Yu et al. 2002 [23]
SD rats	iv lard oil (SFA[b]) or soybean oil (UFA[b]) vs saline for 4.5 h	–	↓ SFA, UFA (HEC)	↓ Akt Px SFA, UFA	n.a.	↑ SFA, UFA	↑ SFA	n.a.	Holland et al. 2007 [52]
Wistar rats	iv Intralipid 12 %[c] vs glycerol for 5 h	–	↓ (HEC)	↑IRS1 tyrPx ↑ Akt Px	↑ (5 h)	↔	↔	n.a.	Hoy et al. 2009 [53]
Wistar rats	HFD, LC-SFA[d] or MC-SFA[e] vs LFD[f] for 8 w	↔ BW ↔ energy intake	↔ LC-SFA ↓ MC-SFA; (GTT+IB)	↔ Akt Px	↑ (LC-SFA)	↔	n.a.	↑ LC-SFA	De Vogel-van den Bosch, et al. 2011 [54]
SphK1 mice	HFD (SFA, MUFA, PUFA mix[f]) vs chow for 6 w	n.a.	↑ (ipGTT+HEC)	↔ PKCθ ↑ Akt Px	↑	↑	↓	n.a.	Bruce et al. 2012 [55]
C57Bl/6 mice	HFD (SFA[g]) vs chow for 3 days-16 w	↑ fat mass	↓(HEC)	↓ Akt Px (3 w) ↔ TNFα and IL-6	↑ 3 w	↑ 3 w (16:0, 16:1, 18:1, 18:2)	↑ 3 w		
(C18:0)	n.a.	Turner et al. 2013 [56]							
C3H/HeOuJ mice	HFD (SFA[h]) vs chow for 4 w	↑ BW ↑ fat mass	↓ (GTT, ITT)	↑ pIRS1-Ser^{1101} ↓ Akt Px ↑ membr./cytos. PKCθ/ε	(↑)	↑	n.a.	n.a.	Badin et al. 2013 [57]
C57Bl/6 mice	HFD[i] vs LFD[j] for 12 w	↑ BW	↓ (ipGTT)	↓ Akt Px	n.a.	n.a.	↑ ↓GluCER	n.a.	Chavez et al. 2014 [43]

SD Sprague Dawley, *HEC* Hyperinsulinemic-euglycemic clamp, *iv* (intravenous), *o* (oral), *ip* (intraperitoneal), *GTT* glucose tolerance test, *ITT* insulin tolerance test, *Px* phosphorylation, *BW* body weight, *KD* knock-down, ↓ decreased compared to control, ↑ increased compared to control, ↔ unchanged compared to control, *n.a.* not available

[a] 10 % safflower oil and 10 % soybean oil

[b] 20 % kcal from fat

[c] Diluted with saline from Intralipid 20 %

[d] 47 % kcal from fat, 75 % C16:0, 6 % C18:0, 13 % C18:1

[e] 47 % kcal from fat, 37 % C8:0, 55 % C10:0

[f] 42 % kcal from fat; SFA (42.7 %), MUFA (35.1 %), PUFA (21.7 %)

[g] 42–45 % kcal from fat, lard

[h] 45 % kcal from fat, mainly SFA: 225 kcal soybean oil, 1598 kcal lard

[i] 60 % kcal from fat, 32.2 % SFA, 35.9 % UFA, 31.9 % PUFA

[j] 10 % kcal from fat

Table 2 Interventional studies in humans: lipid-mediated insulin resistance (IR) in skeletal muscle

Volunteers (n)	Intervention	Insulin sensitivity (method)	Insulin signaling	Intramyocellular lipids				Ref.
				TAG	DAG	CER	ACC	
Healthy male	iv Liposyn II (UFA)[a] vs INS for 6 h	↔ (2 h) ↓ (6 h) (HEC)	↑ PKCβII, PKCδ (6 h) ↓ IκBα (6 h)	n.a.	↑ (6 h)	↔	n.a.	Itani et al. 2002 [25]
Healthy male Healthy female	iv Intralipid 20 % (UFA)[b] vs saline for 7 h[c]	↓ (38 % male) ↓ (24 % female) (HEC)	↔ PI3K ↔ Akt Px	↔	↔	↔	n.a.	Hoeg et al. 2011 [58]
Healthy lean	iv Intralipid 20 % (UFA)[b] for 6 h vs po Sojola (UFA)[d] vs iv LPS vs glycerol for 6 h	↓ (60 %, iv) ↓ (67 %, po) ↓ (48 %, LPS) (HEC)	↑ PKCθ (iv, po) ↔ PKCθ (LPS)	n.a.	↔ total DAG ↑ mDAG[e] (iv)	↔	n.a.	Nowotny et al. 2013 [59]
IR obese T2DM Healthy young	iv Intralipid 20 % (UFA)[b] for 6 h (controls)	↓ (4 h) ↓ (IR, T2DM) (HEC)	↑ PKCθ (IR, T2DM, iv) ↑ pIRS1-Ser^{1101} (4 h) ↓ Akt Px (4 h) ↓ PI3K (4 h)	n.a.	↑ (2.5 h, IR, T2DM) ↑ mDAG. cDAG (iv[f]) ↑ mDAG (T2DM) ↑ cDAG (IR, T2DM)	↔	↔	Szendroedi et al. 2014 [60]
Healthy lean	iv Intralipid 20 % (UFA)[b] vs saline for 48 h	↓ (HEC)	↓ IRS1 tyrPx ↔ Akt Px	n.a.	↔	↔	↔	Hussey et al. 2014 [61]
Healthy trained Healthy sedentary	iv Intralipid 20 % (UFA)[b] vs glycerol for 6 h	↓ (HEC)	n.a.	↔ ↑ C18:2, C18:3	↑ (sedentary[g])	↔	↔	Chow et al. 2014 [62]
Healthy male	iv Intralipid 20 % (UFA)[b] for 1 h+4 h HEC vs baseline	↓ (HEC)	n.a.	n.a.	n.a.	↑	n.a.	Straczkowski et al. 2004 [63]
Healthy male Healthy female	PA-diet (SFA)[h] vs OA-diet (UFA)[i] for 3 weeks Control diet for 7 days	↑ (OA) (ivGTT)	↔ Akt Px (female) ↔ IRS1 Px (female) ↓ JNK (OA, female) ↑Akt Px (OA, male) ↔ JNK Px (male)	↑ (OA, female) ↔ (male)	↔ (female) (↑) (OA, male)	↔ (female) ↑ (PA, male)	↑ MC (PA, female) ↔ (male)	Kien et al. 2013 [64]

HEC Hyperinsulinemic-euglycemic clamp, *iv* (intravenous), *o* (oral), *GTT* glucose tolerance test, *ITT* insulin tolerance test; po: oral fat, *Px* phosphorylation, *cDAG* cytosolic DAG, *mDAG* membrane DAG, *OA* oleic acid, *PA* palmitoic acid, ↓ decreased compared to control. ↑ increased compared to control, ↔ unchanged compared to control, *n.a.* not available

[a] 10 % safflower oil and 10 % soybean oil

[b] 20 % soybean oil

[c] All patients received a controlled diet for 8 days before the experiment

[d] 61 % PUFA, 23 % MUFA, 16 % SFA

[e] C18:2/C18:2

[f] Increased membrane DAG: C18:1/C16:0, C16:0/C20:4, C18:2/C18:0, C18:1/C18:0, C18:1/C18:1, C18:1/C18:2, C18:2/C18:2, C16:0/C18:2, increased cytosolic DAG: C16:0/C20:4, C18:2/C18:0, C18:1/C18:1, C18:1/C18:2, C18:2/C18:2, C16:0/C18:2

[g] Increased DAG species: C18:1, C18:2, C18:3

[h] 40.4 % kcal from fat, 16 % palmitate, 16 % oleate

[i] 40 % kcal from fat, 2.4 % palmitate, 28.8 % oleate

Table 3 Cross-sectional studies in humans: lipid-mediated insulin resistance (IR) in skeletal muscle

Volunteers (*n*)	Insulin sensitivity (method)	Insulin signaling	Intramyocellular lipids				Ref.
			TAG	DAG	CER	ACC	
Obese IR Healthy lean	↓ (obese) (HEC)	↓ Akt Px	n.a.	n.a.	↑ (obese)	n.a.	Adams et al. 2004 [65]
Obese/overweight Lean T2DM-OFF Healthy lean	↓(obese, OFF) (HEC)	n.a.	↑ (obese) ↔ (OFF)	↑ (obese) ↔ (OFF)	↑ (obese) ↑ (OFF)	n.a.	Straczkowski et al. 2007 [66]
Male T2DM Male IGT Healthy Healthy ET	↓ (IGT, T2DM) ↑ (ET) (HEC)	n.a.	↔	n.a.	↔	n.a.	Skovbro et al. 2008 [67]
Obese without T2DM Obese with T2DM	↓(obese without T2DM) (oGTT)	n.a.	↑	↔	n.a.	n.a.	Anastasiou et al. 2009 [68]
Obese Healthy lean	↓ (obese) (HEC)	n.a.	↑ (obese)	↑ C18:1 (obese)	↑ saturated (obese)	n.a.	Moro et al. 2009 [69]
Obese female Healthy lean, female	↓ (obese) (HOMA-IR)	↑ PP2A (obese) ↔ JNK ↔ PKCθ, α, ε	n.a.	↔	↑ mostly C20:0 and C20:4	n.a.	Thrush et al. 2009 [70]
Trained Healthy lean sedentary	↑ (trained) (Minimal model)	n.a.	↑ (trained)	↓ saturated DAG ↔ total DAG	n.a.	n.a.	Bergman et al. 2010 [71]
Obese IR female Obese IS female	↓(IR) (HEC)	n.a.	↑ (type I fibers)	↔	↑ mostly C16:0	n.a.	Coen et al. 2010 [72]
Obese Sedentary ET	↑ (ET) ↓ (obese) (HEC)	n.a.	↑ (ET) ↑ (obese)	↑ (ET[a]) ↑ (obese)	↔ (ET) ↑ (obese[b])	n.a.	Amati et al. 2011 [73]
Class I obese female (31.1 kg/m^2) Class II obese female (45.6 kg/m^2) Healthy female	↓ (CII obese) (HOMA-IR)	n.a.	↑ (obese)	↔	↑ C20:1; C22:1; C24; C24:1 (CII), C14 (CI)	n.a.	Coen et al. 2013 [74]
Obese T2DM ET	↑ ET (ivGTT)	↔ PKCε, PKCθ (ET)	n.a.	↑[c] (obese, T2DM[d])	n.a.	n.a.	Bergman et al. 2012 [104]

HEC Hyperinsulinemic-euglycemic clamp, *GTT* glucose tolerance test, *ITT* insulin tolerance test, *IGT* glucose intolerant, *ET* endurance trained, *IS* insulin sensitive, *Px* phosphorylation, *ETA* endurance trained athletes, *OFF* offspring, ↓ decreased compared to control, ↑ increased compared to control, ↔ unchanged compared to control, *n.a.* not available

[a] C16:0/C18:0 and C16:0/C18:1

[b] Species containing two UFA

[c] Species elevated in T2DM

[d] Species elevated in T2DM: C18:0/C20:4, C16:0/C16:0, C18:0/C18:0

indicating that de novo CER synthesis is exclusively induced by SFA. Moreover, inhibition of CER synthesis prevented from lard oil, but not soybean oil-induced IR. Thus, SFA and UFA induce muscle IR by distinct pathways.

Although HFD induces obesity and IR in rodents [81], the underlying mechanisms are not fully understood. This could be a consequence of a dietary composition, in that diets rich in SFA or UFA could lead to preferential accumulation of CER or DAG. Indeed, SFA induced IR via TLR4-mediated increase in CER [33]. Accordingly, HFD containing similar levels of SFA and UFA induced IR with concomitant increase in both CER and DAG levels [55]. Furthermore, genetic ablation of CER synthesis led to lower CER, higher Akt activity, but only partial protection from HFD-induced IR. Thus, it is conceivable that DAG mediate the residual IR in these mice.

Not all studies support the concept that SFA and TLR4 are exclusively responsible for ceramide-induced IR. For example, TLR4-deficient mice on SFA-rich HFD developed glucose intolerance [82]. Furthermore, SFA-rich HFD increased intramyocellular DAG, PKCθ, and PKCε activation and impaired insulin signaling in mice [57]. CER were not measured, thus, their possible role cannot be excluded. However, rats on SFA-rich HFD also showed 125 % higher intramyocellular DAG, but unchanged CER [83]. On the other hand, mice on HFD rich in either lard or safflower oil (*n*−6 PUFA) had increased CER but no alterations in DAG [84].

These inconsistencies may result from different study designs, i.e., lipid infusion vs. HFD, acute vs. chronic intervention, or different species and strains used. Of note, there is no diet containing exclusively either SFA or UFA. Also, different bioavailability and metabolism of dietary fat likely affects circulating and intracellular lipid class, composition and/or intracellular localization. Mice developed muscle IR after 3 weeks on HFD, but only DAG containing 16:0, 16:1, 18:1, 18:2, and CER C18:0 were increased [56]. Interestingly, 13 weeks later, the DAG and CER profiles changed, underlining the relevance of time-dependent changes for understanding the cause-consequence relationships. In this context, onset of IR due to excessive endogenous lipolysis and elevated FA associated with increases in DAG containing at least one UFA, while CER remained unchanged [85]. As to FA chain length, only saturated MC-FA but not LCFA decreased glucose tolerance in rats independent of impaired insulin signaling [54]. LCFA rather increased intramyocellular ACC but not DAG [54].

Regarding other lipid species, *ob/ob* mice and ZDF rats showed higher levels of muscle GM3 [86, 87], but dietary intervention led to high GluCER in adipose rather than muscle tissue [43]. Moreover, in vitro treatment with GluCER/GM3 induced IR, whereas inhibition of GluCER/GM3 synthesis prevented from IR in adipocytes, but not myocytes. On the other hand, in vivo pharmacological and genetic inhibition of GM3 in mice enhanced insulin signaling and prevented from HFD-induced IR in muscle [87, 88]. Thus, GM3 could be involved in muscle IR and may serve as a target to for its treatment. Finally, lipids such as *n*−3 FA [50], palmitoleate [49] and PASHA in adipose tissue [48] may even positively associate with glucose tolerance and insulin sensitivity in animals.

Humans Liposyn II infusion led to increased serum FA with subsequent IR, characterized by impaired glucose transport/phosphorylation followed by decreased glycogen synthesis in healthy [75, 79] and in T2DM humans [89]. Also, muscle DAG increased with concomitant activation of PKCβII and PKCδ at 6 h, while CER remained unchanged [25]. Interestingly, IκB-α, the inhibitor of NFκB, was reduced, suggesting a link between PKC activation and NFκB-signaling. Indeed, PKCδ binds to TIRAP, a TLR4 downstream adaptor protein, thereby inducing activation of IR-associated kinases such as IKK, MAPK, JNK, and expression of pro-inflammatory factors in human monocytes [90] (Fig. 2). This mechanism could be also relevant in myocytes, where TLR4 and related genes are expressed [91] (Table 2).

Intralipid 20 % infusion also resulted in IR in most studies, frequently associated with changes in DAG but not other lipid species [59, 60, 62]. As in rodents, individual DAG species and their compartmentalization better correlate with the onset of lipid-induced IR. Membrane C18:2/C18:2 [59] and C18:1, C18:2, and C18:3 [62] DAG species accumulated in human muscle after lipid infusion. By performing serial muscle biopsies, we recently described the sequence of events determining the onset of lipid-induced IR [60]. At 2.5 h, both membrane and cytosolic DAG (mostly 18:0, 18:1, and 18:2) were transiently increased, while CER and ACC remain unchanged. At 4 h, PKCθ and pIRS1-Ser1101 increased and PI3K and Akt phosphorylation decreased. These results underlie the presence of dynamic changes in the individual components of lipid-induced IR and the importance of proper timing when designing such experiments. Mild and prolonged (48 h) increase in plasma FA resulted in IR and TLR-related response, without changes in DAG, CER, and ACC [61]. Likely, this study missed an early rise in DAG, while the subsequent effect of inflammation was detected. Similarly, at 5 h of lipid infusion, total DAG and CER were not increased, neither in males nor in females [58]. Another study employing 20 % Intralipid reported increases in total and several species of CER along with IR [63]. However, this study had a time lag of 1 week between baseline and post-intervention biopsies and did not measure DAG. Finally, training status may determine the role of lipid species in lipid-induced IR. In contrast to sedentary humans, IR may dissociate from the muscle DAG accumulation upon lipid infusion in healthy trained males [62].

Lipid infusion may concentration-dependently decrease glucose transport/phosphorylation as demonstrated by an impaired rise in intramyocellular glucose-6-phosphate (G6P)

during fasting peripheral insulinemia with euglycemia (~2.5 h) followed by hyperglycemia [92] as well as hyperinsulinemia with euglycemia (~1 h) [93]. During this time period (~1 h), which precedes the rise in muscle DAG, lipid oxidation is already increased, while glucose oxidation decreased [75, 76], suggesting the additional mechanisms. Operation of the classical Randle cycle postulating that increased FFA availability will compete with glucose for mitochondrial oxidation and thereby give rise to G6P [94] is highly unlikely because of the lack of G6P increase during lipid infusion vs. control conditions [5]. However, mitochondrial emission of reactive oxidative species (ROS) is higher in skeletal muscle of obese and acutely increases in lean 4 h after HFD [95]. Furthermore, short-term reduction in circulating lipids by hypolipidemic agent Acipimox improved insulin sensitivity, decreased whole-body lipid oxidation and reduced ROS production and FFA-linked oxidative capacity in the muscle of T2DM patients [96]. Increased ROS could directly contribute to lipid-induced IR in skeletal muscle, by impairing the insulin signaling via several mechanisms [97]. However, studies exploring time-dependent changes in ROS production and their relationship to lipid oxidation during lipid infusion in humans are missing. In lean rats, lipid infusion induces IR along with concomitant stimulation of ROS production and NFkB signaling [98].

Oral administration of UFA-rich soybean oil-induced IR to similar degree as intravenous Intralipid 20 % infusion [59]. At 5 h after the lipid drink, muscle PKCθ increased by 50 % indicating activation by DAG. Not only CER, but also DAG were unchanged at 5 h [59], likely due to a transient increase in DAG, as discussed above for the Intralipid 20 % infusion studies [60]. Importantly, gender determines the response to different HFD in humans. Oleic acid-rich HFD improved insulin sensitivity and associated with increased TAG levels in women [64]. SFA-rich HFD increased CER pools in men, while MC-ACC were elevated in women and correlated inversely with insulin sensitivity. However, others found only a trend for increased ACC in obese men and women 5 days after HFD, compared to lean controls [99]. Thus, interventional human studies show that specific DAG species and/or their compartmentalization play a critical role in lipid-induced IR.

Cross-sectional studies

Groups with normal insulin sensitivity were compared to those with different degrees of IR or T2DM (Table 3).

Rodents In rodents, either targeted mutagenesis and/or dietary interventions are prerequisite to induce obesity and T2DM. Strain-dependent differences in the susceptibility to HFD-induced metabolic defects and lipid accumulation were compared in five inbred mouse strains [100]. In response to HFD, BL/6, 129X1, DBA/2, and FVB/N mice developed glucose intolerance, but only FVB/N accumulated muscle DAG and CER. Additionally, BALB/c mice remained glucose tolerant and had a trend to lower muscle DAG. Another study confirmed the preferential increase in muscle DAG in FVB compared to BL/6 mice in response to HFD [101]. Thus, the genetic background can indeed determine the tissue-specific metabolic response to dietary interventions.

Humans Intramyocellular TAG, DAG, and CER were increased in obese insulin resistant compared to healthy lean [69]. All lipids were increased also in obese glucose tolerant as well as intolerant humans, but only CER were significantly elevated in male first-degree relatives of patients with T2DM [66]. Nevertheless, both DAG and CER independently predicted IR in these groups. In contrast, CER, but not DAG were higher only in obese females [70, 72], pointing to gender-specific differences. Another study also found higher CER in obese insulin resistant humans [65], but DAG were not reported. Some studies showed that mainly CER with C21:1, C20:4, C20:0, C18:0, and C16:0 FA rather than total CER associated with IR [69, 70, 72]. Even more, total CER in sedentary [67] and certain DAG species in muscle of athletes [66, 73] correlated positively with insulin sensitivity in two studies (Table 3).

Recently, we demonstrated that IR clearly associates with muscle DAG accumulation in obese and T2DM patients, while CER and ACC remained unchanged [60]. Only the cytosolic, but not membrane DAG fraction was increased in obesity, while both fractions were higher in T2DM. Moreover, only certain DAG species and PKCθ translocation clearly correlated with IR. After adjustment for BMI, correlations were strongest for membrane fractions of UFA-containing DAG (C18:1, C18:2, C20:4) [60]. Of note, this exactly mirrors the observations in skeletal muscle of lipid-infused healthy humans, indicating that lipid infusion experiments may indeed reflect the pathophysiological condition of common IR [60]. DAG with at least one UFA are better activators of PKC [21, 102]. But also SFA-rich DAG correlated with IR in persons with the so-called metabolic syndrome [103]. Similar findings were observed in obese, T2DM, and endurance-trained humans [104]. Insulin sensitivity was decreased in obese and T2DM humans and correlated negatively with membrane DAG, which correlated positively with PKCε activity. Particularly, the saturated DAG (C18:0/C20:4, C16:0/C16:0, C18:0/C18:0) were increased in obesity and T2DM [104].

It has been considered a paradox that endurance-trained highly insulin-sensitive humans also have increased levels of IMCL [105] (Table 3). Compared to sedentary T2DM, athletes have more type I fibers, which in general contain more lipids [106] but are characterized by higher glucose-handling capacity [107]. Trained humans exhibited increased total DAG, saturated DAG and DAG containing one UFA, which

correlated positively with muscle insulin sensitivity, while obesity was associated with DAG species comprising two UFA [73]. In one study, endurance-trained individuals had similar DAG levels but lower degree of DAG saturation than healthy sedentary humans [71]. Furthermore, unsaturated and saturated CER were increased in obese, but not in sedentary lean and athletes. In contrast, other trained athletes had higher CER than glucose-intolerant persons; unfortunately DAG were not assessed [67].

These studies underline that total lipid accumulation per se is not prerequisite to induce IR. More likely, subcellular distribution of specific DAG and/or CER as well as muscle fiber composition determines the interference with insulin signaling.

Lipid-induced IR: different fat, different pathways?

Extensive studies in rodents provided novel insights into mechanism of lipid-mediated IR, particularly because they allowed invasive studies of tissue-specific modulation of metabolic pathways. However, the results are heterogeneous in certain aspects, likely due to differences in animal models, type, dosage, and duration of interventions. In humans, divergent data not only result from studying different populations, but rather from limitations to monitor time-dependent changes in various tissues. Common to all studies are differences in the analysis and reporting of lipid metabolites. In this context, absence of changes in total lipid contents does not exclude marked changes in specific lipid metabolites. Also, their intracellular distribution between membrane or cytosolic fractions or lipid droplets might affect their interference with insulin action. Regardless of the experimental differences and techniques, there is compelling evidence that lipid metabolites play a pivotal role in the development of IR. The different lipids not only directly inhibit insulin signaling, but also may stimulate pro-inflammatory pathways, alter mitochondrial function and raise ROS production [97], which in turn accelerates IR.

In human muscle, the current data indicate that both SFA and UFA can stimulate the DAG-PKC pathway to induce IR, while exclusively, SFA activate the fetuin-TLR4-CER pathway. Specifically, UFA-enriched DAGs, such as C18:1 and C18:2 DAGs, rapidly impair proximal insulin signaling via nPKC. Nevertheless, more research is needed to address the crosstalk between these and other pathways and the interference with other known or yet unidentified lipid metabolites. Understanding of the tissue-specific features of lipid-induced IR will be important for further exploiting lipids as target for future strategies to treat patient with IR and T2DM.

Acknowledgments The authors' work is supported by the Ministry of Science and Research of the State of North Rhine-Westphalia (MIWF NRW), the German Federal Ministry of Health (BMG) and by grants of the Federal Ministry for Research (BMBF) to the German Center for Diabetes Research (DZD e.V.), the Helmholtz Alliance with Universities (Imaging and Curing Environmental Metabolic Diseases, ICEMED), the German Research Foundation (DFG; SFB 1116, B05) and the Schmutzler Stiftung, Germany. O.R. was supported by the research training group VIVID of Heinrich-Heine-University.

Conflict of interest The authors declare no conflict of interests.

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