Published in final edited form as:
Med Hypotheses. 2008 ; 70(3): 625–629. doi:10.1016/j.mehy.2007.03.044.

Intramyocellular lipids: maker vs. marker of insulin resistance

ZengKui Guo, Ph.D.
Endocrine Research Unit, Mayo Foundation, Rochester, MN 55905

Abstract

Fifteen years ago it was discovered that intramyocellular triglyceride (imcTG) content in skeletal muscle is abnormally high in lipid oversupply models and, later, in obesity, type 2 diabetes (T2D) and other metabolically diseased conditions. The imcTG abnormality was also found to be significantly correlated with muscle insulin resistance (MIR). As skeletal muscle is the main site for insulin-mediated glucose utilization, the research on this topic has been active since. However, to date the pathways responsible for the imcTG excess and the mechanisms underlying the imcTG-MIR correlation have not been identified. The current view is focused on a backward theory that fatty acid oxidation by muscle is impaired causing imcTG to accumulate. Therefore, an enlarged imcTG pool is merely a marker of MIR and thus is considered a non-player in the development and intervention of MIR. However, it is more likely that imcTG is a source of MIR. On one hand, an enlarged and fast turning over imcTG pool interferes with insulin signaling by producing excess amounts of signaling molecules that activate PKC pathways. On the other hand, it may promote mitochondrial β-oxidation that suppresses glucose metabolism via substrate competition. Therefore, it is hypothesized that imcTG is a source and contributor to MIR.

INTRODUCTION

Intramyocellular triglycerides (imcTG) is an indispensable energy source for skeletal muscle and thus plays pivotal roles in substrate metabolism not only for the tissue but also the whole body given its massive representation (40% of body wt in humans). More than 15 years ago, Storlien and associates discovered that imcTG is elevated by high fat feeding and muscle insulin sensitivity is inversely correlated with imcTG content (r=−0.86–0.93) [1]. This metabolic abnormality has been repeatedly reported for other models as well such as obesity type 2 diabetes, hypertension and etc [2,3,4,5,6,7]. As skeletal muscle is the main site for insulin-mediated glucose uptake and thus a key determinant of whole body insulin sensitivity for glucose metabolism, the implication of the imcTG-MIR correlation to health and diseases cannot be overestimated. Therefore, there is no wonder that the investigations on this issue have been very active. This is exemplified by the establishment and utilization of proton NMR spectroscopy technology for non-invasive measurement of imcTG content [8,9,10,11] that started in the early 1990s [12]. The research primarily involved the mechanisms underlying the imcTG-MIR relation, such as the role of glucose-fatty acid cycling (Randle cycle) and signal transduction in MIR. It was shown that long chain acyl CoA (LCACoA), diacylglycerol (DAG) and ceramides in myocytes cause MIR via interfering with insulin signaling [13,14,15]. In

Correspondence: ZengKui Guo, 5-194 Joseph, Mayo Foundation, Rochester, MN 55905, Phone: (507) 255-6461, Fax: (507) 255-4828, Email: E-mail: guo.zengkui@mayo.edu.

contrast, there have been much less research efforts to study the role of imcTG kinetics, such as imcTG turnover, in MIR.

THE HYPOTHESIS: imcTG as a contributor to MIR

It is hypothesized that enlarged imcTG pool in metabolic diseases is not only a marker of, but a significant and substantive contributor to, MIR. imcTG exerts the effects by releasing large amounts of fatty acids which interfere with insulin signaling via signal transduction pathways. The increased intracellular fatty acid availability also suppresses glucose metabolism by promoting their oxidation by skeletal muscle.

EVALUATION AND DISCUSSION

imcTG turnover

In general, turnover kinetics of imcTG has been scarcely investigated, letting alone for MIR or any metabolic conditions specifically. Nonetheless, limited reports indicated that in resting healthy humans, imcTG pool turns over slowly with a fractional rate of 0.0026/min (calculated from a rate of 29 h/pool based on monexponential e^{-kt}) [16]. In relatively young and healthy men and women exercising at 45% of VO2max, imcTG turned over at a rate of 0.0032±0.0007/min [17]. As expected, imcTG turns over in rodents was much faster as we observed. In lean rats, imcTG fractional turnover rate (FTR) was determined to be 0.013±0.005/min, 0.016 ±0.005/min and 0.0072±0.003%/min for gastrocnemius, tibialis anterior and soleus muscle, respectively. By comparison, FTR of imcTG in high fat-fed obese rats is markedly accelerated in gastrocnemius (0.026±0.002/min, P=0.02) and tibialis anterior (0.030±0.002/min, P=0.01) [18]. The findings are in contrast to the view that imcTG is a static lipid pool turning over slowly causing it to accumulate [19]. Our observations pointed to the contrary that imcTG is a rather dynamic lipid pool with an enlarged pool size. The implication is that the combination of a large pool size and rapid turnover translates into marked increases by a factor of several folds in the absolute turnover and thus release of imcTG-fatty acids. The large amounts of fatty acids released from imcTG increase intracellular fatty acid availability in myocytes. Indeed, in addition to large imcTG pool, we constantly observed increased intramyocellular non-esterified fatty acids in obese rats compared to lean control (gastrocnemius by 70%, soleus by 89% and extensor digitorus longus by 106%, P<0.01). Under the action of acyl CoA synthase, long chain fatty acids are activated to acyl CoA. Long chain fatty acids participate in a number of metabolic pathways including mitochondrial β-oxidation and signal transduction pathways involving PKC, among others.

imcTG oxidation

As imcTG is a local energy source for muscle, an immediate fate for long chain acyl CoA released from imcTG is oxidation in mitochondria to produce ATP. Peroxisomal β-oxidation is minor as it is mainly for shortening very long fatty acids for further oxidation by mitochondria [20,21]. Increased fatty acid availability can accelerate mitochondrial β-oxidation as high substrate concentration enhances enzymatic reactions (mass action). The close vicinity between imcTG droplets and mitochondria in myocytes [22] facilitates this process and thus enhances mitochondrial β-oxidation. Increased fatty acid oxidation is known to interfere with glucose metabolism via the mechanism of substrate competition (Randle cycle, [23]), a widely accepted theory with a large body of literature supporting [24,25,26,27] although it appear not applicable to some conditions such as exercises [28]. Our preliminary results confirmed this that imcTG-palmitate oxidation is significantly higher in high fat-fed obese rats than in lean control (unpublished data). This is consistent with reports

imcTG-fatty acid signaling

Fatty acids must be activated (thioacylated) to acyl CoA before any further steps of metabolism. As intracellular fatty acid flux and availability increase as a result of rapid imcTG turnover, the thioacylation reaction can be enhanced by mass action alone. This is consistent with the higher concentration of long chain acyl CoA in skeletal muscle of obese rats (above). As well known, long chain acyl CoA and diacylglycerol activate PKC (especially θ and ε isoforms) which in turn phosphorylates serine/threonine residues of insulin receptor (IR) and insulin receptor substrate-1 (IRS-1) [13,29]. Phosphorylation of these residues inhibits activation of these proteins causing impairment of insulin signaling and thus insulin-mediated glucose uptake (i.e. MIR).

Meanwhile, imcTG hydrolysis during turnover also releases diacylglycerol such as 1,2-diacylglycerol [30] (triacylglycerol → diacylglycerol + fatty acid), a classical second messenger that acts on the same signaling pathway in a similar manner [19]. Thus, one diacylglycerol and one acyl CoA molecule are produced from the hydrolysis of each imcTG molecule. Both can activate the PKC system to inhibit insulin signaling. In the high fat-fed obese rat model, the content of diacylglycerol in gastrocnemius, soleus and extensor digitorus longus is 52% ($P<0.01$), 37% ($P<0.05$) and 88% ($P<0.01$) higher than that in lean littermate control. And diacylglycerol content is significantly correlated with imcTG pool size and imcTG turnover rate ($r=0.68$, $P<0.05$). The results strongly suggest a precursor-product relationship between imcTG and diacylglycerol. This has been confirmed by using ^{14}C-glycerol as a tracer to track the transition from imcTG to diacylglycerol in rat skeletal muscles using pulse-chase technique (unpublished data).

Elevated long chain acyl CoA and diacylglycerol in conditions of increased lipid supply and insulin resistance have been extensively reported [14, 31, 13, 32, 15]. By comparison, the roles of increased imcTG pool size and accelerated imcTG turnover have not been directly studied and this area of research has not been given much attention. Therefore, the extent of imcTG contributing to the production of these signaling molecules is unknown. For example, what is the relative contribution compared to that from plasma fatty acids. The information is directly relevant to a potential causal role of imcTG in MIR. If a significant part of intramyocellular long chain acyl CoA or/and diacylglycerol is derived from imcTG, then it can be concluded that imcTG substantively contributes to MIR. In other words, imcTG is a contributor to, and not merely a marker of, MIR. This can be confirmed or verified experimentally by using ^{14}C/^{13}C-glycerol to trace the production of diacylglycerol from imcTG and ^{14}C/^{13}C-fatty acid isotopes to trace the release of acyl CoA by using pulse-chase technique (to prelabel the precursor pool first and then following the label flow to the product pools).

Fatty acids are also a precursor to ceramides, yet another member of the signaling molecule family with similar functions. Thus, increased fatty acid availability stimulates ceramide synthesis (e.g. palmitoyl ceramide). This further worsens the impairment of insulin signaling.

Taken together, long chain acyl CoA, diacylglycerol and ceramides can all be produced from imcTG. When imcTG pool is enlarged, the fluxes are increased. Rapid imcTG turnover amplifies this mass effect by dramatically increasing the rate of efflux of fatty acids and diacylglycerol from imcTG and thus local fatty acid trafficking and availability. As a result, excess amounts of these signaling molecules are produced and they can jointly activate PKC system. MIR results (Fig 1).

This mechanism can be further intensified with reduced fatty acid oxidation when more fatty acids become available for signaling. On the other hand, even increased fatty acid oxidation may not necessarily divert fatty acids away from PKC pathways to a degree that reverses the mechanism because the large fatty acid efflux from imcTG may suffice to maintain the needs

for both pathways simultaneously. In such case, glucose utilization by muscle suffers dual suppressions, one being impaired insulin-mediated uptake as a result of impaired insulin signaling and the other being reduced glucose uptake via substrate competition due to increased fatty acid oxidation. This may well be the case as the evidence is overwhelming that muscle lipid oxidation in obesity and type 2 diabetes is elevated [24, 33, 34, 25, 35, 36, 37, 38, 39, 40]. Our recent data showed the similar observation that mitochondrial β-oxidation in skeletal muscle of high fat-fed obese rats is greater than that in lean control (unpublished data). Since the obesity model has also accelerated imcTG turnover [18] and elevated diacylglycerol content (above), it appears that these indices of lipid hyper-metabolism co-exist in skeletal muscle of obesity and this supports the hypothesis.

On the other hand, impaired lipid oxidation by skeletal muscle has also been observed [41, 19]. It is possible that certain metabolic conditions may modify the relationship among these parameters. For example, over weight and non-extreme obesity have higher but extreme obesity has lower lipid oxidation in muscle [37]. The lack of reliable methodologies for measuring muscle lipid oxidation in vivo is another factor. For example, often the difference between total lipid oxidation (indirect calorimetry) and plasma fatty acid oxidation (fatty acid tracer) is used to represent lipid oxidation by muscle while it also includes oxidation by other tissues. Therefore, the issue of muscle lipid oxidation requires further investigation with more reliable techniques. Nonetheless, there seem no doubts that intramyocellular signaling molecules of PKC system are high in obesity and other metabolic conditions. As impairment of insulin signaling may be more powerful in suppressing glucose metabolism, this factor alone is powerful enough to cause MIR.

SUMMARY

Ultimate proof of increases in mitochondrial β-oxidation and in the fluxes of imcTG-fatty acids to PKC signaling pathways co-existing with MIR will question the validity of the marker theory. This will reverse the previous backward mechanism to a forward one where imcTG is a contributor to MIR rather than a marker. Such a reversal is fundamental to the understanding and intervention of MIR. For example, it will become a target for intervention of MIR, rather than a surrogate.

Acknowledgments

The work is supported by NIH research project R01 grant DK 60013 and ADA Research Award 7-05-RA-48. The author thanks Dr. Michael D. Jensen for providing access to research resources. The technical services provided by Lianzhen Zhou (Ph.Lic.) are appreciated used to obtain the data as mentioned herein.

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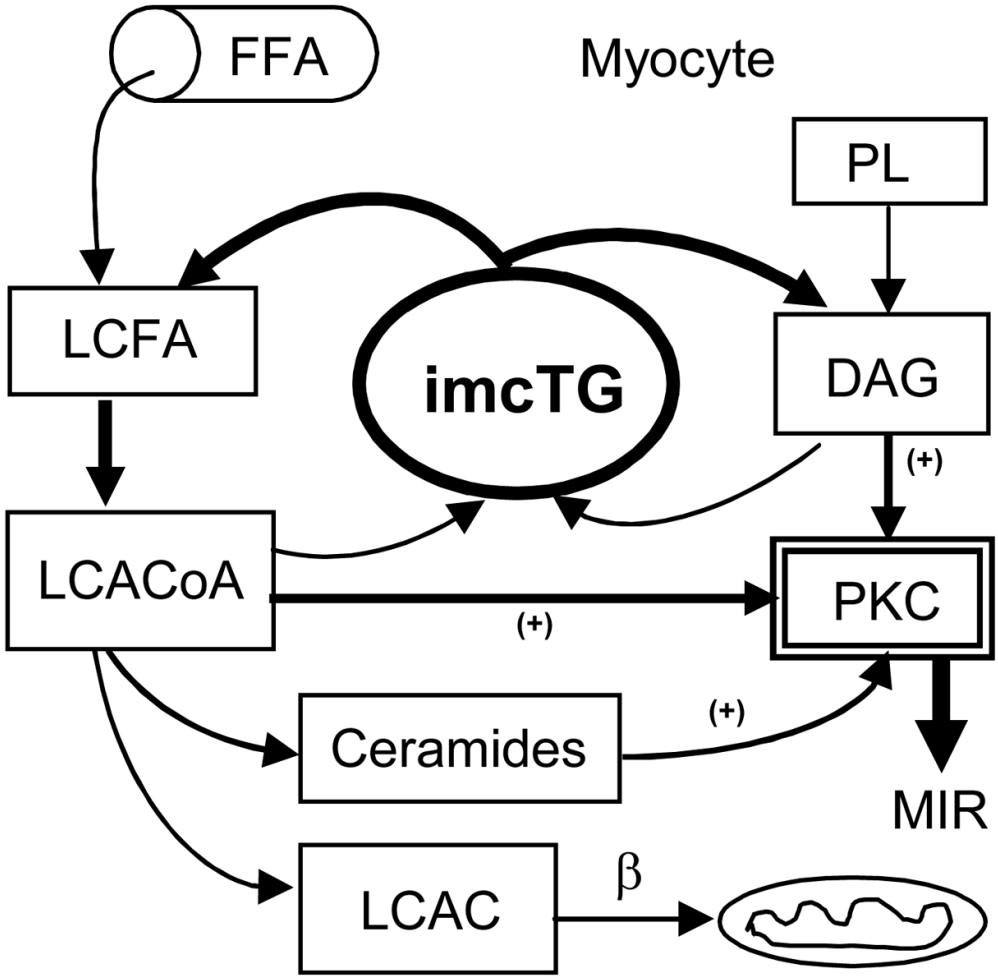

Figure 1.

Hydrolysis of imcTG produces DAG and LCACoA which is also precursor to ceramides. All three can activate PKC and thus inhibit insulin signaling. A large, rapid turning over imcTG pool increases their production and thus MIR results. This continues even at increased lipid oxidation given the large imcTG-fatty acid flux and reduced mito β-oxidation worsens it. FFA, (plasma) free fatty acids; LCFA, long chain fatty acids; LCACoA, long chain acyl CoA; LCAC, long chain acylcarnitines; PL, phospholipids; DAG, diacylglycerol; PKC, protein kinase C; β, mitochondrial β-oxidation.