CROSSTALK

Comments on CrossTalk 47: Intramuscular lipid accumulation causes/does not cause insulin resistance

PKC is not a one-trick pony

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This Crosstalk debate (Goodpaster, 2020) firstly highlights the widely reported association between accumulation of intramuscular lipid intermediates, such as diacylglycerols and ceramides, and development of muscle insulin resistance. Secondly, however, it is clear that insulin resistance can arise for different reasons, and under certain conditions, a causative relationship is not supported. It was agreed that further investigation into different mechanisms and their clinical importance is required. In this regard, emphasis is now frequently placed on the importance of defining the species and subcellular localization of diacylglycerols and ceramides that modulate insulin action. Although important, on its own this may merely refine the existing correlations to a higher resolution. Demonstration of causation also requires mechanistic interventions downstream of lipid accumulation. Previous work in muscle and other insulin target tissues has typically concentrated on interference with insulin signal transduction, such as the phosphorylation of IRS-1 or the insulin receptor itself by diacylglycerol-activated protein kinase C (PKC). This focus needs to be broadened: PKC-mediated insulin receptor phosphorylation has been challenged (Brandon *et al.* 2019) and muscle insulin resistance may in large part be independent of proximal signalling defects (Czech, 2017). Pertinently, while PKCθ activation is frequently linked to muscle insulin resistance, its role extends beyond insulin signalling, encompassing neuromuscular junction remodelling (Lanuza *et al.* 2014), physical activity and energy expenditure (Gao *et al.* 2007). In addition, as lipid sensors, PKCs are well-placed to mediate feedback upon lipid metabolism itself, as evidenced by several *in vivo* studies of PKC-deficient mice exhibiting lipid abnormalities (Schmitz-Peiffer, 2013).

References

Brandon AE, Liao BM, Diakanastasis B, Parker BL, Raddatz K, McManus SA, O'Reilly L, Kimber E, van der Kraan AG, Hancock D, Henstridge DC, Meikle PJ, Cooney GJ, James DE, Reibe S, Febbraio MA, Biden TJ & Schmitz-Peiffer C (2019). Protein kinase C epsilon deletion in adipose tissue, but not in liver, improves glucose tolerance. *Cell Metab* **29**, 183–191.e7.

Czech MP (2017). Insulin action and resistance in obesity and type 2 diabetes. *Nat Med* **23**, 804–814.

Gao Z, Wang Z, Zhang X, Butler AA, Zuberi A, Gawronska-Kozak B, Lefevre M, York D, Ravussin E, Berthoud HR, McGuinness O, Cefalu WT & Ye J (2007). Inactivation of PKCθ leads to increased susceptibility to obesity and dietary insulin resistance in mice. *Am J Physiol Endocrinol Metab* **292**, E84–E91.

Goodpaster BH (2020). CrossTalk proposal: Intramuscular lipid accumulation causes insulin resistance. *J Physiol* **598**, 3803–3806,

Lanuza MA, Santafe MM, Garcia N, Besalduch N, Tomàs M, Obis T, Priego M, Nelson PG & Tomàs J (2014). Protein kinase C isoforms at the neuromuscular junction: localization and specific roles in neurotransmission and development. *J Anat* **224**, 61–73.

Schmitz-Peiffer C (2013). The tail wagging the dog - regulation of lipid metabolism by protein kinase C. *FEBS J* **280**, 5371–5383.

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Intramuscular lipid accumulation and insulin resistance: influence of a noisy neighbour

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We read with interest the cross-talk articles on intramuscular lipids (IMCL) and muscle insulin resistance. While the time course of IMCL accumulation and insulin resistance is disconnected during disuse (Dirks *et al.* 2020), studies in high-fat-fed rodents show that the accumulation of triacylglycerol (TAG), diacylglycerols and ceramides in muscle is intimately linked with the onset of impaired insulin-stimulated glucose uptake (Turner *et al.* 2013). Although not evidence of causation, this demonstrates the onset of muscle insulin resistance is temporally linked with IMCL accumulation. Conducting similar studies in humans to capture the onset of pathological insulin resistance is challenging but would provide further insight into this relationship.

Methodological issues in assessing IMCL also present challenges. For example, there were conflicting results regarding IMCL use during exercise, as many studies using biochemical extraction methods failed to show exercise-induced TAG degradation (Watt *et al.* 2002). This was attributed to high variability due to contamination from neighbouring intermuscular adipocytes and/or fibre type differences. This was largely overcome by using ^{1}H-magnetic resonance spectroscopy (Krssak *et al.* 2000) or immunofluorescence microscopy (van Loon *et al.* 2003). These techniques have consistently shown exercise-induced reductions in IMCL which are specific to type I muscle fibres. Intermuscular adipocytes are highly prevalent in insulin-resistant populations and will influence biochemical assessments of IMCL content and composition. The application of novel sophisticated methods to assess lipid content, composition and location will provide further clarity on the role of specific IMCL species in mediating muscle insulin resistance.

References

Dirks ML, Wall BT & Stephens FB (2020). CrossTalk opposing view: Intramuscular lipid accumulation does not cause insulin resistance. *J Physiol* **598**, 3807–3810.

Krssak M, Petersen KF, Bergeron R, Price T, Laurent D, Rothman DL, Roden M & Shulman GI (2000). Intramuscular glycogen and intramyocellular lipid utilization during prolonged exercise and recovery in man: a ^{13}C and ^{1}H nuclear magnetic resonance spectroscopy study. *J Clin Endocrinol Metab* **85**, 748–754.

Turner N, Kowalski GM, Leslie SJ, Risis S, Yang C, Lee-Young RS, Babb JR, Meikle PJ, Lancaster GI, Henstridge DC, White PJ, Kraegen EW, Marette A, Cooney GJ, Febbraio MA & Bruce CR (2013). Distinct patterns of tissue-specific lipid accumulation during the induction of insulin resistance in mice by high-fat feeding. *Diabetologia* **56**, 1638–1648.

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van Loon LJ, Koopman R, Stegen JH, Wagenmakers AJ, Keizer HA & Saris WH (2003). Intramyocellular lipids form an important substrate source during moderate intensity exercise in endurance-trained males in a fasted state. *J Physiol* **553**, 611–625.
Watt MJ, Heigenhauser GJ & Spriet LL (2002). Intramuscular triacylglycerol utilization in human skeletal muscle during exercise: is there a controversy? *J Appl Physiol* **93**, 1185–1195.

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Intramyocellular lipids: one of many factors that can lead to insulin resistance

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In this CrossTalk debate the effect of intramuscular lipid (IMCL) accumulation on insulin resistance (IR) is discussed (Dirks *et al.* 2020; Goodpaster, 2020). Similar to the 2016 CrossTalk debate that discussed whether or not intramyocellular ceramide accumulation causes insulin resistance, there are a large number of studies presented from both views supporting or refuting the ability for lipids to cause IR (Petersen & Jurczak, 2016; Summers & Goodpaster, 2016). Despite these discussions, many aspects of the relationship between IMCLs and IR remain unknown – particularly when considering, as stated by both Goodpaster and Dirks *et al.*, there are several causes of IR, with physical inactivity/skeletal muscle disuse representing just one of them.

The studies presented by Dirks *et al.* make a convincing argument that IMCLs do not cause, but may be causal of, IR in models of skeletal muscle disuse (Wall *et al.* 2015). However, physical inactivity may not be representative of the many causes of IR, such as obesity, diet or genetics, and a growing amount of data suggests that it is not. At the very least, strong evidence exists for the ability of specific IMCLs (ceramides, diacylglycerols) to initiate IR, particularly in response to different diets or genetic manipulation (Chaurasia *et al.* 2019). Ultimately, this debate highlights the complexity of insulin resistance, with no silver bullet. Rather, many triggers that mechanistically differ may contribute. While the debate is sure to continue, perhaps we can all agree that the aetiology of insulin resistance is complex, with many factors influencing this pathology.

References

Chaurasia B, Tippetts TS, Mayoral Monibas R, Liu J, Li Y, Wang L, Wilkerson JL, Sweeney CR, Pereira RF, Sumida DH, Maschek JA, Cox JE, Kaddai V, Lancaster GI, Siddique MM, Poss A, Pearson M, Satapati S, Zhou H, McLaren DG, Previs SF, Chen Y, Qian Y, Petrov A, Wu M, Shen X, Yao J, Nunes CN, Howard AD, Wang L, Erion MD, Rutter J, Holland WL, Kelley DE & Summers SA (2019). Targeting a ceramide double bond improves insulin resistance and hepatic steatosis. *Science* **365**, 386–392.
Dirks ML, Wall BT & Stephens FB (2020). CrossTalk opposing view: Intramuscular lipid accumulation does not cause insulin resistance. *J Physiol* **598**, 3807–3810.
Goodpaster BH (2020). CrossTalk proposal: Intramuscular lipid accumulation causes insulin resistance. *J Physiol* **598**, 3803–3806.
Petersen MC & Jurczak MJ (2016). CrossTalk opposing view: Intramyocellular ceramide accumulation does not modulate insulin resistance. *J Physiol* **594**, 3171–3174.
Summers SA & Goodpaster BH (2016). CrossTalk proposal: Intramyocellular ceramide accumulation does modulate insulin resistance. *J Physiol* **594**, 3167–3170.
Wall BT, Dirks ML, Snijders T, Stephens FB, Senden JM, Verscheijden ML & van Loon LJ (2015). Short-term muscle disuse atrophy is not associated with increased intramuscular lipid deposition or a decline in the maximal activity of key mitochondrial enzymes in young and older males. *Exp Gerontol* **61**, 76–83.

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None.

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Intramuscular lipids: new advances reveal greater biological complexity

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The ongoing debate on whether intramuscular lipid accumulation directly causes insulin resistance has been reignited in this crosstalk series. It would be over-simplistic to suggest that insulin resistance is caused exclusively by intramuscular lipids, because skeletal muscle is remarkably adaptable to numerous insults including forced contraction, substrate availability, inflammatory signalling, and intra-organellar stress and dysfunction. Each could contribute to impaired insulin action. The debate as to whether intracellular lipid accumulation in muscle drives insulin resistance, however, continues.

Both Goodpaster and Dirks *et al.*, are in agreement that the association of insulin resistant markers to total lipid content is over-simplistic. New advances in lipid quantification have identified specific lipid species within the glycerol or sphingolipid families that associate with insulin resistance and glucose metabolism (Turpin *et al.* 2014, Turpin-Nolan *et al.* 2019). The focus of research is now shifting to where these specific species are accumulating within intracellular compartments, as highlighted by recent articles focusing upon distinct ceramide species accumulation within the glucoregulatory cells (Hammerschmidt *et al.* 2019, Turpin-Nolan & Bruning 2020).

Goodpaster argues that intramuscular ceramide content is driving insulin resistance. It is important to note, however, that a reduction of skeletal muscle $C_{18:0}$ ceramides by transgenic mouse models (Turpin-Nolan *et al.* 2019) or pharmacological intervention (Turner *et al.* 2018) leads to either improved whole body glucose tolerance or increased skeletal muscle fatty acid oxidation, but not both.

References

Hammerschmidt P, Ostkotte D, Nolte H, Gerl MJ, Jais A, Brunner HL, Sprenger HG, Awazawa M, Nicholls HT, Turpin-Nolan SM, Langer T, Kruger M, Brugger B & Bruning JC (2019). CerS6-derived sphingolipids interact with Mff and promote mitochondrial fragmentation in obesity. *Cell* **177**, 1536–1552.e23.
Turner N, Lim XY, Toop HD, Osborne B, Brandon AE, Taylor EN, Fiveash CE, Govindaraju H, Teo JD, McEwen HP, Couttas TA, Butler SM, Das A, Kowalski GM, Bruce CR, Hoehn KL, Fath T, Schmitz-Peiffer C, Cooney GJ, Montgomery MK, Morris JC & Don AS (2018). A selective inhibitor of

ceramide synthase 1 reveals a novel role in fat metabolism. *Nat Commun* **9**, 3165.
Turpin SM, Nicholls HT, Willmes DM, Mourier A, Brodesser S, Wunderlich CM, Mauer J, Xu E, Hammerschmidt P, Bronneke HS, Trifunovic A, LoSasso G, Wunderlich FT, Kornfeld JW, Bluher M, Kronke M & Bruning JC (2014). Obesity-induced CerS6-dependent C16:0 ceramide production promotes weight gain and glucose intolerance. *Cell Metab* **20**, 678–686.
Turpin-Nolan SM & Bruning JC (2020). The role of ceramides in metabolic disorders: when size and localization matters. *Nat Rev Endocrinol* **16**, 224–233.
Turpin-Nolan SM, Hammerschmidt P, Chen W, Jais A, Timper K, Awazawa M, Brodesser S & Bruning JC (2019). CerS1-derived $C_{18:0}$ ceramide in skeletal muscle promotes obesity-induced insulin resistance. *Cell Rep* **26**, 1–10.e7.

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Intramuscular lipid accumulation causes insulin resistance? Physiological context is essential

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One CrossTalk article (Dirks *et al.* 2020) is focussed on acute muscle disuse in healthy young, volunteers whilst the sister article (Goodpaster, 2020) centres primarily on chronic muscle pathophysiology in human obesity and diabetes. It is unequivocal that whole body and muscle insulin resistance are common end-points of each scenario, but it is highly likely that the aetiology of insulin resistance is very different in each case. Accordingly, each article is faithful to its title, but this simply underlines there are several drivers of muscle insulin resistance. Indeed, without the context of the predominant physiological state and its duration being considered from the outset it is like comparing apples and pears debating whether intramuscular lipid accumulation causes insulin resistance.

References

Dirks ML, Wall BT & Stephens FB (2020). CrossTalk opposing view: Intramuscular lipid accumulation does not cause insulin resistance. *J Physiol* **598**, 3807–3810.
Goodpaster BH (2020). CrossTalk proposal: Intramuscular lipid accumulation causes insulin resistance. *J Physiol* **598**, 3803–3806.

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Flip it and reverse it: paralysis, intramuscular lipids and insulin resistance

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Paralysis as a result of spinal cord injury (SCI) represents a model of chronic muscle disuse, characterised by an imbalance between nutrient supply and utilization. Elder *et al.* (2004) demonstrated a 4-fold greater thigh intramuscular fat (IMF) percentage in individuals with SCI compared to able-bodied controls, along with greater insulin resistance (IR). Jonkers *et al.* (2012) reported no differences in intramyocellular lipid (IMCL) content in participants with paraplegia compared to able-bodied controls, although extramyocellular lipid content was greater in paraplegics and negatively associated with whole body insulin sensitivity (IS). These data, although limited by the cross-sectional design, are in line with the dissociation between IMCL and IR put forth by Dirks *et al.* However, the role of specific IMCL species and their location in the aetiology of IR following SCI remains to be elucidated.

Electrical stimulation of deconditioned, paralysed muscles allows us to understand the importance of muscle contraction as a primary mediator of IR (irrespective of IMCL). A recent systematic review noted improvements in markers of peripheral IS (5/6 studies) following functional electrical stimulation cycling in individuals with SCI (Farrow *et al.* 2020), with Chilibeck *et al.* (1999) revealing a substantial increase in skeletal muscle glucose transporter protein levels (72%) and oxidative capacity (56%), whereas electrical resistance training has demonstrated a negligible (combined with dietary restriction) or no reduction in IMF of paralysed muscles (Gorgey *et al.* 2012; Ryan *et al.* 2013). These data suggest 'improvement' in peripheral IS is not necessarily dependent on a reduction in IMF.

References

Chilibeck PD, Bell G, Jeon J, Weiss CB, Murdoch G, MacLean I, Ryan E & Burnham R (1999). Functional electrical stimulation exercise increases GLUT-1 and GLUT-4 in paralyzed skeletal muscle. *Metabolism* **48**, 1409–1413.
Elder CP, Apple DF, Bickel CS, Meyer RA & Dudley GA (2004). Intramuscular fat and glucose tolerance after spinal cord injury – a cross-sectional study. *Spinal Cord* **42**, 711–716.
Farrow M, Nightingale TE, Maher J, McKay CD, Thompson D & Bilzon J (2020). The effect of exercise on cardiometabolic risk factors in adults with chronic spinal cord injury: A systematic review. *Arch Phys Med Rehabil*, doi: 10.1016/j.apmr.2020.04.020.
Gorgey AS, Mather KJ, Cupp HR & Gater DR (2012). Effects of resistance training on adiposity and metabolism after spinal cord injury. *Med Sci Sports Exerc* **44**, 165–174.
Jonkers RA, Dirks ML, Nabuurs CI, De Feyter HM, Praet SF, Nicolay K, van Loon LJ & Prompers JJ (2012). Myofibrillar distribution of succinate dehydrogenase activity and lipid stores differs in skeletal muscle tissue of paraplegic subjects. *Am J Physiol Endocrinol Metab* **302**, E365–E373.
Ryan TE, Brizendine JT, Backus D & McCully KK (2013). Electrically induced resistance training in individuals with motor complete spinal cord injury. *Arch Phys Med Rehabil* **94**, 2166–2173.

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Are intramyocellular lipids always the culprit of insulin resistance?

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Dr Goodpaster (Goodpaster, 2020) presents compelling evidence supporting a causal role of intramyocellular lipids (IMCL) in the development of insulin resistance (IR). In contrast, Dr Dirks and colleagues (Dirks *et al.* 2020) argue that IMCL do not cause IR because in short-term muscle disuse studies, IR is detected before any increase in IMCL. However, these views are not mutually exclusive. The absence of evidence can be attributed to the experimental conditions (underpower, analytical protocols, timing of the muscle biopsy) rather than to the absence of a biological link. The onset of IR in response to physical inactivity could also initially result from factors other than lipotoxic pressure, i.e. inflammation or unfolded protein response (Petersen & Shulman, 2018). The accumulation of IMCL could appear later and still exacerbate the initial development of IR. In support, we showed that bedrest-induced IR is preceded by the inability of the body to increase fat oxidation in response to a lipid overload (i.e. metabolic inflexibility) (Rudwill *et al.* 2018), which likely leads to IMCL accumulation. Therefore, the fact that IMCL do not *always* cause IR does not mean that they *never* play a causal role in its development. Future research will need to unravel the mechanistic cascade of the complex aetiology of IR and determine the respective role of the potential factors, including IMCL. Measuring specific diacylglycerol and ceramide subspecies according to chain length, saturation, stereospecificity and intracellular location will further help refine our understanding of the intricate relationship between lipotoxic pressure and IR.

References

Bergouignan A, Trudel G, Simon C, Chopard A, Schoeller DA, Momken I, Votruba SB, Desage M, Burdge GC, Gauquelin-Koch G, Normand S & Blanc S (2009). Physical inactivity differentially alters dietary oleate and palmitate trafficking. *Diabetes* **58**, 367–376.

Dirks ML, Wall BT & Stephens FB (2020). CrossTalk opposing view: Intramuscular lipid accumulation does not cause insulin resistance. *J Physiol* **598**, 3807–3810.

Goodpaster B (2020). CrossTalk proposal: Intramuscular lipid accumulation causes insulin resistance. *J Physiol* **598**, 3803–3806,

Petersen MC & Shulman GI (2018). Mechanisms of insulin action and insulin resistance. *Physiol Rev* **98**, 2133–2223.

Rudwill F, O'Gorman D, Lefai E, Chery I, Zahariev A, Normand S, Pagano AF, Chopard A, Damiot A, Laurens C, Hodson L, Canet-Soulas E, Heer M, Meuthen PF, Buehlmeier J, Baecker N, Meiller L, Gauquelin-Koch G, Blanc S, Simon C & Bergouignan A (2018). Metabolic inflexibility is an early marker of bed-rest-induced glucose intolerance even when fat mass is stable. *J Clin Endocrinol Metab* **103**, 1910–1920.

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Determining a causal effect of intramyocellular lipid accumulation on insulin resistance in humans will require new experimental approaches

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The authors on both sides of this CrossTalk debate have made many valuable contributions to the literature that have improved the understanding of associations between intramyocellular lipid (IMCL) accumulation and insulin resistance (IR). Findings from physical inactivity studies that IR precedes significant alterations in IMCL accumulation show that increased IMCL are not *required* for the induction of IR in this paradigm (Dirks *et al.* 2020). However, this does not eliminate the possibility that IMCL cause IR under other conditions. Goodpaster (2020) cites recent cross-sectional studies reporting correlations between IMCL species in specific cellular compartments and markers of IR to support a causal role for IMCL on IR. Although these cross-sectional studies have undoubtedly moved the field forward and highlighted the need to consider the subcellular location of IMCL (Chung *et al.* 2017; Perreault *et al.* 2018), their designs preclude causal inference. Longitudinal experimental approaches to manipulate the abundance (and/or localization) of specific IMCL species while keeping other key variables reasonably constant are needed. It will be inherently challenging to pin exercise- or weight loss-induced improvements in IR on IMCL modifications due to the pleiotropic nature of these interventions. Perhaps novel pharmacological approaches will enable the manipulation of IMCL with minimal 'off-target' effects in humans. Currently, while the debate over causality of IMCL accumulation on IR in humans is certainly interesting, we seem to be lacking an experimental approach that could definitively answer the question.

References

Chung JO, Koutsari C, Blachnio-Zabielska AU, Hames KC & Jensen MD (2017). Intramyocellular ceramides: subcellular concentrations and fractional de novo synthesis in postabsorptive humans. *Diabetes* **66**, 2082–2091.

Dirks ML, Wall BT & Stephens FB (2020). CrossTalk opposing view: Intramuscular lipid accumulation does not cause insulin resistance. *J Physiol* **598**, 3807–3810.

Goodpaster BH (2020). CrossTalk proposal: Intramuscular lipid accumulation causes insulin resistance. *J Physiol* **598**, 3803–3806.

Perreault L, Newsom SA, Strauss A, Kerege A, Kahn DE, Harrison KA, Snell-Bergeon JK, Nemkov T, D'Alessandro A, Jackman MR, MacLean PS & Bergman BC (2018). Intracellular localization of diacylglycerols and sphingolipids influences insulin sensitivity and mitochondrial function in human skeletal muscle. *JCI Insight* **3**, e96805.

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