Consequences of an obesogenic diet can be prevented by knockout of P2Y₆ purinergic receptor in mice

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Article summary

Obesity is a precursor to an array of pathologies ranging from insulin resistance, to serious health complications like cardiovascular diseases. Elucidating the discrete cellular signalling pathways underpinning metabolic health is critical to understanding and tackling these obesity-driven health concerns. For instance, the role of intracellular Ca²⁺ signalling in adipocytes is still poorly understood and much of existing literature concerning receptors modulating intracellular Ca²⁺ offer contradicting outcomes. In a recent *PNAS* publication [1] have illustrated that subversion of Gq-linked purinergic receptor P2Y₆ signalling results in significant reductions of body mass and inflammation, alongside significant improvements in glucose tolerance and insulin sensitivity. The positive effects of global P2Y₆ receptor knockout were significant enough to override receptor absence in skeletal muscle, where P2Y₆ receptor facilitates glucose uptake independent of insulin stimulation. This demonstrates the bimodal, nonlinear role of purinergic receptors in metabolism and research should rightfully be directed at delineating purinergic P2 receptor contribution in the adipose context. The positive metabolic effects observed were due to loss of P2Y₆-mediated JNK signalling. Concomitantly, the authors illustrate how P2Y₆-mediated JNK activity is required to maintain the white adipocyte phenotype through suppression of thermogenic genes such as peroxisome proliferator-activated receptor alpha (*Ppara*). Consequently, their research advocates the use of purinergic antagonists as therapies against obesity-associated diseases.

Commentary

The ever-increasing prevalence of obesity and its associated suite of pathologies, e.g. type II diabetes and cardiovascular disease (among a litany of others) causes us to face economic, social and healthcare costs that may eventually impede our ability to treat the disorder effectively. This reality is driving research in understanding the complex intracellular signalling mechanisms underpinning our normal and pathological states of metabolism, ultimately for the generation of targeted therapeutics against obesity-associated diseases.

It is widely cited that plasma levels of inflammatory markers such as C-reactive protein, interleukins and nucleotides such as ATP and UDP are increased in obese individuals [2]. This is likely due to excessive caloric intake and subsequent fat storage driving white adipose tissue (WAT) remodelling, which disrupts normal signalling patterns and results in both local and systemic inflammation [2]. Purinergic receptors possess the ability to be activated by circulating nucleotides and elicit a plethora of context-dependent cellular responses via Ca²⁺ signalling or altering adenylyl cyclase activity [3]. Of these purinergic receptors, evidence has shown the active role members of the metabotropic P2Y GPCR family have in aspects of metabolic regulation, e.g. lipolysis regulation [4]. However, there is limited scientific evidence on the mechanistic outcome of intracellular Ca²⁺ signalling in adipocytes. Given the reported abundance of receptors that modulate intracellular Ca²⁺ in adipocytes [4], alongside the disparity between publications on the outcome each receptor has, it's critical that definitive evidence is generated.

A case in point, previous work by the Jacobson group demonstrated P2Y₆ receptor increased glucose uptake in adipocyte and skeletal muscle cell lines [5]. Therefore, Jain et al. continued the research to establish how these observations were mechanistically underpinned using in vivo and in vitro analysis in a murine model.

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By imposing a high-fat diet (HFD) challenge on mice, the authors were able to determine the impact diet-induced obesity has on expression of P2Y₆ receptor in metabolically active adipose and skeletal muscle tissues. Interestingly, they observed a fivefold increase in P2Y₆ receptor mRNA expression in adipose tissues, with halved expression in skeletal muscle. To determine whether the upregulation of P2Y₆ receptor was beneficial, whole-body P2Y₆ receptor knockout (KO) mice were generated using a *cre-lox* model. Contrary to their previous findings [5], under HFD regimes, P2Y₆ receptor KO mice had significantly lower mass, improved glucose tolerance (ability to manage glucose load) and sensitivity to insulin stimulation. Interestingly, plasma levels of free fatty acids (FFA) remained unchanged in P2Y₆ receptor-KO animals, indicating P2Y₆ receptor has limited influence on lipolysis. Revealingly, measurements of CO₂ production/O₂ consumption revealed KO mice had significantly higher energy expenditure than controls. The data seem to suggest that P2Y₆ receptor expression not only increases under a HFD regime in adipose tissues, but these increases are detrimental to global metabolic regulation.

Next, skeletal muscle-specific P2Y₆ receptor KO animals were generated to probe the bimodality such receptors possess in different tissues. Under HFD regimes, animals lacking skeletal muscle P2Y₆ receptor suffered from severely impaired glucose tolerance and insulin sensitivity, while expression of mRNA associated with oxidative phosphorylation was downregulated (e.g. *Pgc1α*, *Pparδ*, *Cycs*). Under normal physiological conditions, the selective P2Y₆ receptor agonist MRS4383 enhanced uptake of radio-labelled glucose independent of insulin. Therefore, the positive metabolic affects observed in global KO of P2Y₆ receptor are clearly dominant enough to override impeding affects contributed by loss of receptor in skeletal muscle, and its action in skeletal muscle may potentiate the metabolic improvements seen in adipose-specific KOs.

The authors also considered the role P2Y₆ receptor plays in inflammation, in the context of an obesogenic diet. Effects of both global and adipocyte-specific P2Y₆ receptor knock-out manifested in reduced inflammatory infiltration (macrophages and monocytes) in adipose tissue, confirmed by significant reductions of immune cell and proinflammatory molecule mRNA (e.g. *Mcp1*, *Il6*, *Tnfα*). These anti-inflammatory effects extended systemically through reductions of plasma-circulating IFNγ, MCP1 and resistin, culminating in anti-inflammatory consequences in discrete tissues. For example, in adipose-specific P2Y₆ receptor-KO mice, a reduction in ectopic fat deposition and mRNA of inflammatory markers (*Il6*, *Ifng*, *Mcp1* and *Il1b*) was observed in the liver. These results illuminate the role P2Y₆ receptor may have in the obesity-associated inflammatory phenotype as often described in literature [2].

To identify signalling alterations that confer the physiological benefit, Jain and colleagues established adipocyte cell lines to compare downstream protein changes after P2Y₆ receptor activation. The authors reasoned that resulting G_q-mediated Ca²⁺ increases in adipocytes from P2Y₆ receptor stimulation may activate kinases such as JNK (as is the case in the liver). Thus, their investigation was directed at alterations in the activity of JNK, as it has previously been implicated in the metabolic dysfunction of adipocytes [6]. While the Ca²⁺/JNK relationship is not directly illustrated in their study, they show Western blotting signals for activated JNK (p-JNK) and its downstream effector c-Jun were significantly increased after P2Y₆ receptor-specific activation, while P2Y₆ receptor KO cells lacked this response.

The next natural step was to determine the ultimate outcome of JNK signalling in adipocytes. By examining the enrichment of adipocyte DNA at discrete sites after P2Y₆ receptor-specific activation (ChIP analysis), the authors found substantial enrichment of PPARα promotor regions in a JNK/c-Jun-dependent manner, inhibiting its transcription. The inhibitory enrichment of PPARα promotor regions was absent in P2Y₆ receptor KO animals and removed in *wt* tissues with a JNK-specific inhibitor (SP600125) cementing the P2Y₆ receptor-JNK inhibitory axis. PPARα has featured in many metabolic publications and is typically associated with brown adipose tissue (BAT) and thermogenesis due to its role in FFA trafficking, mitochondrial β-oxidation and correlation with UCP-1 expression [7]. The authors demonstrate that adipose morphology of P2Y₆ receptor-KO animals changes to appear as if 'beiging', as matched by increased browning-related protein expression, including PPARα, UCP1 and PGC1α. Therefore, it's likely that to maintain a white adipose tissue as a storage organ, such thermogenic genes are contextually and constitutively suppressed. Perhaps differential nucleotide availability or purinergic receptor abundance between WAT and BAT allows for a contextual means of suppression to maintain the white adipocyte phenotype. The research by Jain et al. therefore opens the floor for further research to this end.

Purinergic signalling in the context of adipose tissue has not been fully and thoroughly investigated. Examining available literature provides only partial resolution as to the role and consequences of Ca²⁺ signalling within adipocytes. Here, Jain et al. have provided systematic evidence of P2Y₆ receptor-mediated suppression of thermogenic-related genes via JNK signalling in adipocytes, and how it may preserve the white phenotype. Also, by demonstrating the role of adipocyte P2Y₆ receptors as a systemic modulator of glucose tolerance, insulin sensitivity and inflammation, it has highlighted the potential for targeted therapeutics aimed at obesity-associated disorders such as metabolic syndrome and type II diabetes. Especially since adipose P2Y₆ upregulation under obesogenic conditions widens the therapeutic target.

However, as only P2Y₆ receptor-specific agonists were applied, it presents a limiting feature of this study as other G_q-linked P2Y receptors present on adipocytes may converge on the JNK pathway. By way of demonstration, presence of activated JNK and c-JUN was noted in P2Y₆ receptor KO animals, albeit to a lesser extent. A panel of purinergic agonists alongside MRS4383 would have established both the specificity of the P2Y₆ receptor-JNK interaction, while indicating contribution of other P2Y₆ receptor to JNK activation. This may already be underway by Jain and colleagues, as they are industriously developing novel P2Y₆ receptor ligands for future studies.

The next step for the metabolic research community is applying these techniques to other purinergic receptors in adipocytes to expand our knowledge of purinergic contribution in metabolic regulation, and to widen our therapeutic repertoire.

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Declarations

Ethical approval The author declares all animal studies are conducted in accordance with NIH guidelines for Animal Research and approved by NIDDK Institutional Animal Care and Use Committee.

Conflicts of interest The author declares no competing interests.

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