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RAPID COMMUNICATION

Irisin regulates pancreatic lipases through PPARγ-PGCα-FNDC5 pathway

Irisin, a secreted myokine actively cleaved from fibronectin type III domain-containing protein 5 (FNDC5), plays a crucial role in whole-body metabolism. To date, irisin receptor has not been identified, and its signal transduction is mostly unknown. Irisin is regulated by peroxisome proliferator-activated receptor-gamma (PPARγ) coactivator-1-alpha (PGC1α).[1] We previously showed that PPARγ directly regulates pancreatic lipase (PL), the principal dietary lipolytic enzyme that plays an essential role in dietary fat digestion, linking dietary fat to PL regulation.[2] Pancreatic lipase-related protein 2 (PLRP2) sharing high homology with PL is also produced and secreted by the pancreas, although in different expression patterns and with different substrate specificity.[3] Irisin and PL share specific nutrient regulation (fatty acids) and partial signal transduction pathway,[2,4] yet the role of irisin in exocrine pancreas function is unknown. We aim to study FNDC5/irisin role in the exocrine pancreas functionality.

Short-term treatment (4 h) with exogenous irisin (60 ng/ml) significantly ($P < 0.05$) suppressed PPARγ-PGC1α-FNDC5 transcripts (by 2, 2.2, and 1.9-fold, respectively) and protein expression levels (by 1.9, 1.7, and 1.4-fold, respectively) in exocrine pancreas acinar AR42J-B13 cells, resulting in significant down-regulation of both pancreatic lipases: PL and PLRP2 at transcript (by 2.5 fold), protein expression (by 5 and 2.3-fold, respectively) (Fig. 1A, B), and secretion levels *in vitro* (by 2.8 and 1.5-fold, respectively) (Fig. 1C, D). Irisin inhibitory effect through the PPARγ pathway was also demonstrated by using PPARγ overexpression (OE-PPARγ) AR42J-B13 cells, where exogenic irisin significantly reduced PPARγ-PGC1α-FNDC5 transcripts (by 1.8, 1.7, and 1.8-fold, respectively) and protein expression levels (by 1.3, 1.4, and 1.6-fold, respectively), and PL and PLRP2 transcripts (by 3.7, and 1.7-fold, respectively) and protein expression (by 6.5, and 3.6-fold, respectively) along with PL and PLRP2 secretion levels (by 1.3, and 1.5-fold, respectively) (Fig. 1A–D). Significant suppression effect of irisin on pancreatic lipase secretion was further demonstrated by using an *ex-vivo* rat primary acinar cell model, an acceptable model for digestive enzyme secretion, where exogenous irisin treatment significantly decreased the secreted PL and PLRP2 (by 2.9, and 3.7-fold, respectively) compared to untreated cells (Fig. 1E, F). In contrast to irisin, treatment with rosiglitazone (rosi, 100 nM), a pharmacological agonist of PPARγ, significantly increased the expression of the PGC1α-FNDC5 axis at both transcript (by 1.4, and 1.9-fold, respectively) and protein levels (by 2.2, and 1.8-fold, respectively), as well as PL and PLRP2 transcripts (by 2.6, and 3.5-fold, respectively), protein expression (by 1.6, and 1.8-fold, respectively) (Fig. 1G, H), and secretion levels (1.5, and 1.7-fold, respectively) compared to control cells (Fig. 1I, J). Similarly, OE-PPARγ AR42J-B13 cells exhibited a significant up-regulation of the PPARγ-PGC1α-FNDC5 axis transcripts (by 6023, 3.9, and 6.2-fold, respectively) and protein levels (by 1.7, 2.2, and 2.1-fold, respectively), as well as PL and PLRP2 transcripts (by 17 and 12-fold, respectively) and protein expression levels (by 3.2, and 3.5-fold, respectively), and PL secretion level (by 1.6-fold) (Fig. 1G–J). PPARγ and its co-activator PGC1α act as nuclear receptors and transcription factors. Upon activation, PPARγ and PGC1α translocate to the nucleus and stimulate gene expression.[2] Both rosi treatment and OE-PPARγ resulted in a significant translocation of both PGC1α (red; Fig. 1K, L; Fig. S1A) and PPARγ (green; Fig. 1M, N; Fig. S1B) to the nucleus as demonstrated by immunofluorescent staining, indicating activation of the PPARγ-PGC1α pathway. In contrast, challenge with exogenous irisin significantly decreased PGC1α and PPARγ translocation to the nucleus, further indicating that exogenous irisin has an inhibitory effect on the PPARγ- PGC1α pathway (Fig. 1K, N; Fig. S1A, B). Together, these results suggest that PPARγ-PGC1α pathway is involved in expression of pancreatic lipases and FNDC5 in acinar cells, and indicate an inhibitory effect of exogenous irisin on the PPARγ-PGC1α-FNDC5 signal transduction, which results in an inhibitory effect on pancreatic lipase expression and secretion. Chronic

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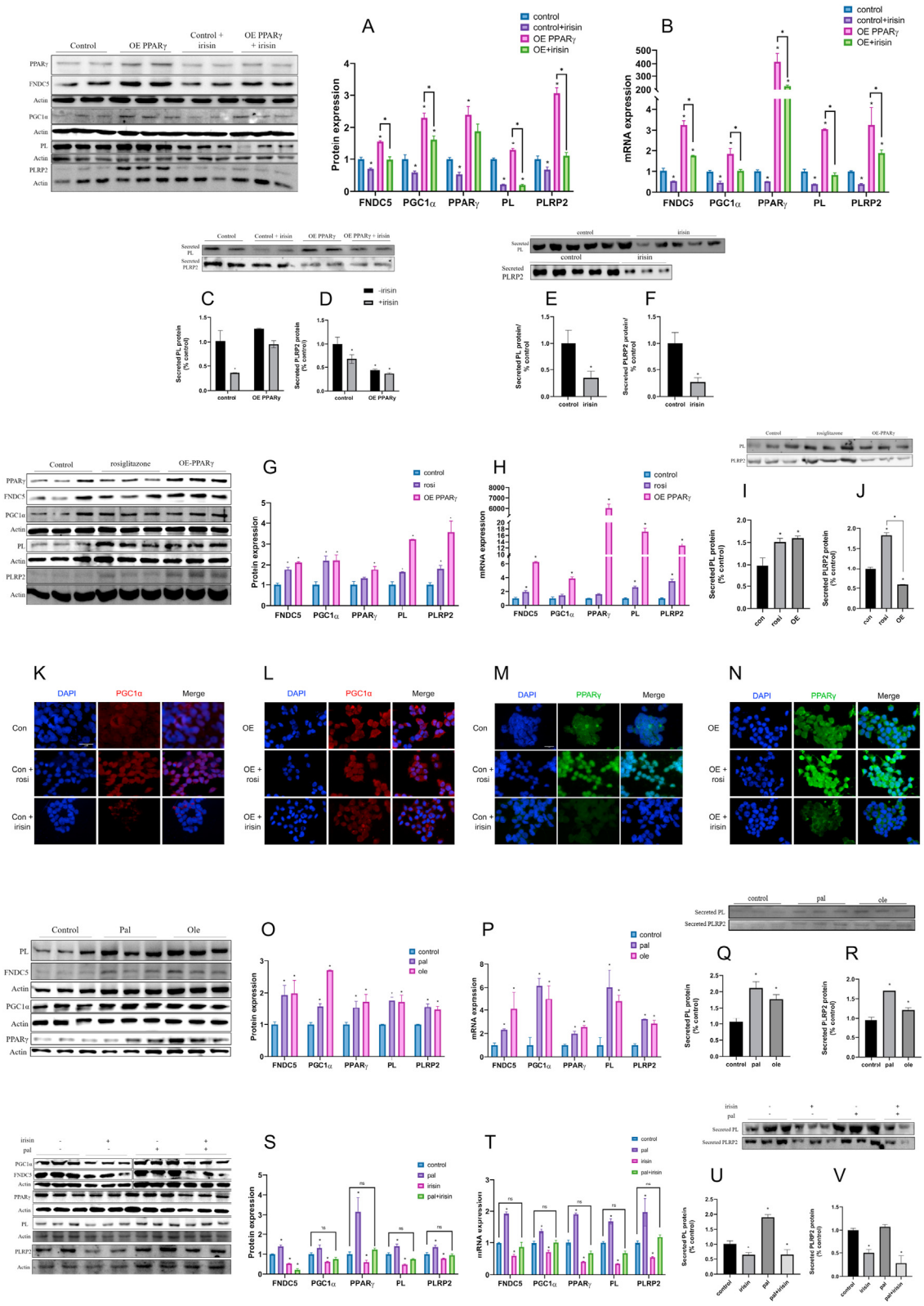

Figure 1 Irisin/FNDC5 is involved in pancreatic lipase regulation via the PPARγ-PGC1α pathway in response to nutrient flux. (**A**) Western blot analysis of protein levels normalized to actin in AR42J-B13 cells (control and OE-PPARγ) treated with exogenous irisin (60 ng/mL, 4 h). **(B)** RT-PCR analysis of transcript levels normalized to S18 in AR42J-B13 cells (control and OE-PPARγ) treated with exogenous irisin (60 ng/mL, 4 h). **(C)** Secreted PL protein levels in AR42J-B13 cells (control and OE-PPARγ) treated with exogenous irisin (60 ng/ml, 4 h). **(D)** Western blot analysis of secreted PLRP2 protein levels in AR42J-B13 cells (control and OE-PPARγ) treated with exogenous irisin (60 ng/mL, 4 h). **(E)** Western blot analysis of secreted PL protein levels in primary exocrine pancreas acinar cells were treated with exogenous irisin (60 ng/mL, 4 h). **(F)** Western blot analysis of secreted PLRP2 protein levels in primary

hyperglycemia is the main feature characterizing NIDDM (Non-insulin-dependent diabetes mellitus), including in acinar cells proximity. Irisin is responsive to glucose levels and is reported to be involved in glucose homeostasis in several tissues, particularly the endocrine pancreas.[1] We have recently shown crosstalk between the endocrine and exocrine pancreas pathologies, where endocrine pancreas insufficiency is frequent, such as in the state of NIDDM reflected by high-glucose environment, and induced endoplasmic reticulum (ER) stress state in acinar cells.[5] In order to mimic NIDDM environment and stimulate hyperglycemia state, acinar cells were exposed to either low (5.5 mM) or high (25 mM) concentration of glucose. Glucose dose-dependently and significantly up-regulated the FNDC5-PPARγ-PGC1α pathway at the transcript and protein expression levels (Fig. S2A–C), as well as digestive enzyme associated with glucose homeostasis such as amylase (Fig. S2D). These results indicate that glucose induces FNDC5 expression and affects acinar cells' functionality by regulating exocrine pancreas enzyme expression. Irisin/FNDC5 is regulated by fatty acids,[4] and fatty acids are also known regulators of PPARγ and pancreatic lipases.[2] Thus, we investigated the alterations of FNDC5-PPARγ-PGC1α pathway and pancreatic lipases in response to different fatty acids (FAs): palmitic acid (Pal) and oleic acid (Ole). Exposure of exocrine pancreas cells to both FAs resulted in significant increased FNDC5-PPARγ-PGC1α axis in parallel to significant up-regulation of PL and PLRP2 transcripts (by 6, 3.2-fold, respectively), protein expression levels (by 1.8, and 1.6-fold, respectively) (Fig. 1O, P), and secretion levels (by 2.1, and 1.7-fold, respectively) in Pal-treated cells compared to control cells (Fig. 1Q, R). Pal is the most abundant FA in the body. Additionally, irisin was shown to highly responsive to Pal.[4] In order to further demonstrate the direct inhibitory role of irisin on PL, we studied the combined synergistic effect of Pal (24 h) and irisin (4 h) treatments in AR42J-B13 cells on PPARγ-PGC1α-FNDC5 and acinar cells functionality. Pal treatment significantly up-regulated PPARγ-PGC1α-FNDC5 pathway, and expression and secretion of PL. Exogenous irisin had a significant inhibitory effect on PPARγ-PGC1α-FNDC5, and pancreatic lipase expression (Fig. 1S, T) and secretion (Fig. 1U, V). The combination of Pal and irisin treatment restored PPARγ-PGC1α-FNDC5, and transcript and protein expression levels of PL and PLRP2 to similar levels measured in the untreated control cells. Combined Pal and irisin treatment significantly reduced secretion levels of pancreatic lipases to the levels following irisin treatment alone (Fig. 1S–V). Our novel findings showed that FNDC5 was expressed in the exocrine pancreas and was regulated by the PPARγ-PGC1α axis. Exogenous irisin had a significant inhibitory effect on the PPARγ-PGC1α-FNDC5-axis regulating the expression and nuclear translocationof PPARγ and PGC1α. Irisin inhibitory effect resulted in a significant reduction in acinar cell functionality, as demonstrated by decreased synthesis, expression and secretion of PL and PLRP2, by using *in vitro* and *ex vivo* models. FNDC5/irisin expression was shown to response to dietary glucose and FA. Irisin is a known metabolic regulator of lipid and glucose metabolism, inducing energy expenditure, weight loss and body fat reduction.[1,4] To our knowledge, our results demonstrate, for the first time, the positive effect of glucose levels on PPARγ-PGC1α induction in the exocrine pancreas acinar cells, further augmenting the dietary effect on FNDC5 expression. Pancreatic lipases are regulated by the amount of dietary fat, and directly activated by PPARγ.[2] Reduced activity of pancreatic lipases is related to fat malabsorption, and as a consequence, body fat reduction and weight loss.[2] Our current results suggest that irisin inhibits pancreatic lipase activation and secretion possibly via the restrictive effect on PPARγ-PGC1α axis, leading to decreased fat digestion and thus contributing to irisin's

exocrine pancreas acinar cells were treated with exogenous irisin (60 ng/mL, 4 h). **(G)** Western blot analysis of protein levels normalized to actin in AR42J-B13 cells (control and OE-PPARγ) treated with rosiglitazone (100 nM, 24 h). **(H)** RT-PCR analysis of transcript levels normalized to S18 in AR42J-B13 cells (control and OE-PPARγ) treated with rosiglitazone (100 nM, 24 h). **(I)** Western blot analysis of secreted PL protein levels in AR42J-B13 cells (control and OE-PPARγ) treated with rosiglitazone (100 nM, 24 h). **(J)** Western blot analysis of secreted PLRP2 protein levels in AR42J-B13 cells (control and OE-PPARγ) treated with rosiglitazone (100 nM, 24 h). **(K)** Immunohistochemistry staining of PGC1α (red) and DAPI nucleus staining (blue) of AR42J-B13 acinar cells following treatment with rosiglitazone (24 h) or irisin (4 h). **(L)** Immunohistochemistry staining of PGC1α (red) and DAPI nucleus staining (blue) of OE-PPARγ AR42J-B13 acinar cells following treatment with rosiglitazone (24 h) or irisin (4 h). **(M)** Immunofluorescent staining of PPARγ (green) and DAPI nucleus staining (blue) of AR42J-B13 acinar cells following treatment with rosiglitazone (24 h) or irisin (4 h). **(N)** Immunofluorescent staining of PPARγ (green) and DAPI nucleus staining (blue) of OE-PPARγ AR42J-B13 acinar cells following treatment with rosiglitazone (24 h) or irisin (4 h). Photos were taken using Olympus fluorescent microscope at × 60 magnification (1 μm scale). **(O)** Western blot analysis of protein levels normalized to actin in AR42J-B13 cells treated with fatty acid (Pal, Ole, 500 μM) for 24 h. **(P)** RT-PCR analysis of transcript levels normalized to S18 in AR42J-B13 cells treated with fatty acid (Pal, Ole, 500 μM) for 24 h. **(Q)** Western blot analysis of secreted PL protein levels in AR42J-B13 cells treated with fatty acid Pal, Ole, 500 μM) for 24 h **(R)** Western blot analysis of secreted PLRP2 protein levels in AR42J-B13 cells treated with fatty acid (Pal, Ole, 500 μM) for 24 h. **(S)** Western blot analysis of protein levels normalized to actin in AR42J-B13 cells treated with Pal (500 μM, 24 h), exogenous irisin (60 ng/mL, 4 h) or combed treatments. **(T)** RT-PCR analysis of transcript levels normalized to S18 in AR42J-B13 cells treated with Pal (500 μM, 24 h), exogenous irisin (60 ng/mL, 4 h) or combed treatments. **(U)** Western blot analysis of secreted PL protein levels in AR42J-B13 cells treated with Pal (500 μM, 24 h), exogenous irisin (60 ng/mL, 4 h) or combed treatments. **(V)** Western blot analysis of secreted PLRP2 protein levels in AR42J-B13 cells treated with Pal (500 μM, 24 h), exogenous irisin (60 ng/mL, 4 h) or combed treatments. Results of RT-PCR and Western blot are expressed in the graphs as mean ± SE of 3–4 independent experiments ($n = 3$). Immunofluorescence staining was performed in quadruplet for each experiment, and quantification of PPARγ and PGC1α nuclear localization was performed on 200–250 cells for each treatment (randomly chosen). Asterisks represent statistical difference from control. *$P < 0.05$.

known effect on weight loss. Our results imply that FNDC5 may act as a link between dietary nutrients, the exocrine pancreas function and digestion regulation contributing to whole-body metabolism.

Conflict of interests

The authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.03.017.

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