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Irisin modulates genes associated with severe coronavirus disease (COVID-19) outcome in human subcutaneous adipocytes cell culture

Miriane de Oliveira[a,*], Maria Teresa De Sibio[a], Lucas Solla Mathias[a], Bruna Moretto Rodrigues[a], Marna Eliana Sakalem[b], Célia Regina Nogueira[a]

[a] Department of Internal Clinic, Botucatu Medicine School, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil
[b] Department of Anatomy, Londrina State University (UEL), Londrina, Parana, Brazil

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ABSTRACT

Obesity patients are more susceptible to develop COVID-19 severe outcome due to the role of angiotensin-converting enzyme 2 (*ACE2*) in the viral infection. *ACE2* is regulated in the human cells by different genes associated with increased (*TLR3*, *HAT1*, *HDAC2*, *KDM5B*, *SIRT1*, *RAB1A*, *FURIN* and *ADAM10*) or decreased (*TRIB3*) virus replication. RNA-seq data revealed 14857 genes expressed in human subcutaneous adipocytes, including genes mentioned above. Irisin treatment increased by 3-fold the levels of *TRIB3* transcript and decreased the levels of other genes. The decrease in *FURIN* and *ADAM10* expression enriched diverse biological processes, including extracellular structure organization. Our results, in human subcutaneous adipocytes cell culture, indicate a positive effect of irisin on the expression of multiple genes related to viral infection by SARS-CoV-2; furthermore, translatable for other tissues and organs targeted by the novel coronavirus and present, thus, promising approaches for the treatment of COVID-19 infection as therapeutic strategy to decrease *ACE2* regulatory genes.

1. Introduction

Obesity is the most common metabolic disorder in the world, and is a public health problem that affects both developed and developing countries (GBD, 2015 Obesity CollaboratorsAfshin et al., 2017; D 2015 Eastern Mediterr, 2018; Mokdad et al., 2016). The amount and distribution of adipose tissue is associated with many adverse consequences, such as hypertension, type II diabetes, cardiovascular diseases (PHAbraham et al., 2019), and may lead to decreased expiratory reserve volume (Venkata et al., 2010).

The emergence and spread of 2019 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the possible development of coronavirus disease (COVID-19) it brings, has led us to a public health crisis, threatening the world and its population, since and all groups and ages are susceptible (Singhal, 2020). Infected patients may never develop any symptoms; and the ones affected by COVID-19 may present variable clinical conditions, from loss of smell and taste, to mild respiratory issues, and with the possibility of severe symptoms such as respiratory insufficiency, that can result in death (Singhal, 2020). The SARS-CoV-2 virus particles are transmitted by symptomatic or asymptomatic carrier people, and in the case of people developing COVID-19, the propagation begins days before the onset of symptoms, in form of large droplets during coughing and sneezing (Rothe et al., 2020), and possibly even during normal conversations, what results in a disseminated mass contagion (Stadnytskyi et al., 2020). An individual is infected by inhaling viral droplets or by touching a contaminated surface and then the face mucous membranes in the nose, mouth or eyes (World Health Organization, 2020).

In face of this scenario, of a highly infective disease with potential to cause death, it has been previously shown that people with a pre-existing chronic medical condition such as obesity are more susceptible to develop severe COVID-19 outcomes (Chen et al., 2020). Obesity has an impact on pulmonary function as it leads to decreased functional capacity and respiratory system compliance; a fact that may explain the impact of COVID-19 on these patients. Furthermore, increased inflammatory cytokines may contribute to the increased morbidity associated with obesity in COVID-19 infections (Dietz and Santos-Burgoa, 2020).

The more frequent development of severe form of COVID-19 in patients with chronic medical conditions is possibly related to the role of angiotensin-converting enzyme 2 (*ACE2*) in the viral infection (Wang et al., 2020). *ACE2* is widely expressed in the lungs, cardiovascular

* Corresponding author. São Paulo State University (UNESP), Botucatu Medical School, District of Rubião Jr, s/n, 18618, Botucatu, São Paulo, Brazil.
E-mail addresses: miriane.oliveira@unesp.br, miriane.oliveira85@gmail.com (M. de Oliveira).

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system, gut, kidneys, central nervous system, and adipose tissue (Gheblawi et al., 2020), and is the target of the virus since SARS-CoV-2 infects the alveolar epithelial cells of the lung using *ACE2* as an input receptor (Zhou et al., 2020). Adipocytes and adipocyte-like cells, such as pulmonary lipofibroblasts, may play an important role in the pathogenic response to COVID-19, especially in obese and diabetic individuals; in these individuals, the *ACE2* is increased which turns adipose tissue into a potential target and viral reservoir (Kruglikov and Scherer, 2020).

Recently, a molecular study conducted with 700 lung transcriptome samples from patients with comorbidities, cardiovascular diseases, familial primary pulmonary hypertension, hypertension and pulmonary hypertension, associated with severe COVID-19 showed that ACE2 is more expressed in these patients than in control individuals (Pinto et al., 2020). In addition, there are other reports that contain other several genes with potential importance for SARS-CoV-2 cell cycle and invasion/attachment, that have been shown to be up-regulated in patients with severe COVID-19 comorbidities, such as: ADAM metallopeptidase domain 10 (*ADAM10*), toll like receptor 3 (*TLR3*), histone acetyltransferase 1 (*HAT1*), histone deacetylase 2 (*HDAC2*), lysine demethylase 5B (*KDM5B*), sirtuin 1 (*SIRT1*), member RAS oncogene family (*RAB1A*), transmembrane serine protease 2 (*TMPRSS2*), and furin paired basic amino acid cleaving enzyme (*FURIN*) (Pinto et al., 2020). In the case of FURIN, it is a cellular endoprotease that proteolytically activates large numbers of proprotein substrates in secretory pathway compartments, as well as activates pathogenic agents; also, *FURIN* plays an essential role in embryogenesis, and catalyses the maturation of a strikingly diverse collection of proprotein substrates (Thomas, 2002).

In a study with transcriptional analyzes of proteins that interact with SARS-CoV-2, altered transcripts in aging lung were observed, such as increased hyaluronan and proteoglycan link protein 2 (*HAPLN2*), and decreased while tribbles homolog 3 (*TRIB3*) (Moraes et al., 2020). *TRIB3*, mainly expressed in type I (AT1) and type II (AT2) alveolar cells (Madissoon et al., 2019), is probably the common target as a protective factor for other viruses, as it has been previously shown for flavivirus dengue and Zika (Zanini et al., 2018), and the increased replication of hepatitis C virus (HCV) was associated with its inhibition (Tran et al., 2016). *TRIB3* interacts with nucleocapsid protein and RNA-dependent RNA polymerase of human coronavirus (HCoVs) may decrease virus infection and replication (Moraes et al., 2020).

Irisin is a cleaved peptide formed from the fibronectin type III domain-containing protein 5 (*FNDC5*) (Boström et al., 2012), which directs white adipose tissue to brown adipose tissue with increased thermogenesis, which was suggested as a therapeutic agent for human metabolic disease such as obesity (Perakakis et al., 2017). It has been postulated that irisin modulates macrophage activity by reducing reactive oxygen species (ROS) overproduction, which could suggest its potential anti-inflammatory properties, with possible important action also in the course of lung injury (Shao et al., 2017a).

Obesity is a chronic disease, a fact that makes the patients more susceptible to severe infection by SARS-Cov-2, and irisin could have a positive effect on inflammation; in addition, plasma and subcutaneous adipose tissue expression of irisin are decreased in patients with obesity (Moreno-Navarrete et al., 2013; Frühbeck et al., 2020). Based on this, the present study aimed to evaluate the effects of irisin on human subcutaneous adipocytes through global transcriptome analysis, focusing on the genes studied in previous researches (Pinto et al., 2020; Moraes et al., 2020). Through RNA-Seq-based transcriptomic analysis, we provide evidence that the human subcutaneous adipocytes in culture do not express *ACE2* mRNA, but express *ADAM10*, *TLR3*, *HAT1*, *HDAC2, KDM5B*, *SIRT1*, *RAB1A*, *FURIN* and *TRIB3*. Irisin treatment increased by 3-fold the levels of *TRIB3* transcript and decreased the levels of other genes. The RNA-Seq analysis was selected in order to perform a “free” investigation of the genes expressed in the cells, because RNA-Seq does not require predesigned probes, the data sets are unbiased, allowing for hypothesis-free experimental design.

2. Materials and methods

2.1. Culture and cell differentiation

Human subcutaneous preadipocytes (HPAd - 802S-05A; Cell Applications, Inc. San Diego, CA, USA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Preadipocytes were cultured, differentiated in adipocytes and treated with 1 μg/ml (20 nM) irisin (Sigma-Aldrich St. Louis, MO, USA), as previously described (de Oliveira et al., 2020).

2.2. RNA extraction, preparation, sequencing and bioinformatics analysis

Total RNA was extracted from adipocytes using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) followed by preparation to build of the cDNA libraries, sequencing and bioinformatics analysis as previously described (de Oliveira et al., 2020). Differentially expressed (DE) genes were classified as up and down, considering Fold Change (FC) values > 1.3 for the irisin group. Gene Ontology (GO) enrichment analyses for biological processes (BP) were performed with the cluster Profiler package using the p.adjust false discovery rate and $p < 0.05$ were used to demonstrate gene expression from RNA-seq data and interactive graphic was generated by REVIGO (http://revigo.irb.hr/) (Supek et al., 2011). The values are expressed in fragments per kilobase of exon model per million reads mapped (FPKM), and each sample will have one for each gene, were used to demonstrate the expression of genes in adipocytes and modulation of genes by irisin who are out FC. Differentially expressed genes found in the RNA-seq were not validated by real-time PCR in this article data, we believe our data analyzed with RNA-seq needs no further investigation by real-time PCR, since our aim, in this moment, to identify differences in gene expression.

2.3. Statistical analyses

FPKM values of three samples per groups for each gene were analyzed using Student's t-test to compare results between irisin and control groups, after performing the Kolmogorov Smirnov normality test. Data are expressed as mean ± standard deviation. The significance level was set at 5%.

3. Results

3.1. Reduction of FURIN and ADAM10 by irisin leads to the enrichment of several terms in Gene Ontology (GO)

Both *FURIN* and *ADAM10* genes were downregulated after irisin treatment within the FC established in our study. They are known to participate along with other genes downregulated by irisin in the enrichment of 14 and 12 terms in Gene Ontology (GO), respectively, that represent biological processes (Board 1 and 2). Figs. 1 and 2 represent the enriched GO terms, which involve the *FURIN* and *ADAM10* genes, respectively, and show genes grouped by similarity (Similarity = 0.7); the interactive graphics were generated by REVIGO (http://revigo.irb.hr/) (Supek et al., 2011).

3.2. Irisin treatment effect on TLR3, HAT1, HDAC2, KDM5B, SIRT1, RAB1A, FURIN, ADAM10 and TRIB3 mRNA expression

In our study, DE genes were classified as up or downregulated considering FC values > 1.3 for irisin and control groups. Although all *TRIB3, TLR3, HAT1, HDAC2, KDM5B, SIRT1, RAB1A*, *FURIN* and *ADAM10* genes are expressed in adipocytes, only *FURIN* and *ADAM10* were downregulated by irisin within the FC established in our study. However, it is possible to demonstrate the action of irisin in other genes using the values expressed in FPKM. The *TRIB3* transcript level was increased by 3-fold compared to the control group. *TLR3* and *SIRT1*

Table 1
TreeMap analysis for the terms involves FURIN and other genes Down regulated by Irisin. The grouped terms and the enriched genes are represented for each main term found after the TrreMap cluster analysis; 14 terms are grouped into 6 main terms (Representative_term).

Representative_term	Term_description	Term_ID	qvalue	geneID
regulation of lipase activity	regulation of lipase activity	GO:0060191	0,026871	552/10316/5156/22925/1909/2263/148/4023/5159/5125/**5045**/6272/2260/1956
	regulation of hormone levels	GO:0010817	0,001497	338557/6566/3708/3709/775/220/6422/1363/10159/51144/3624/8862/10891/4311/25825/10699/1382/8322/2697/399979/1717/8622/51092/8879/133/54884/5125/84649/**5045**/51109/2260/5959/25976/51714/114897/949/51768/6720/1956/10404/4041/1889/9249/57555
	hormone metabolic process	GO:0042445	0,002596	339761/3952/5156/64577/3077/1545/157506/220/1363/10159/51144/10891/4311/25825/10699/1382/1717/8879/133/54884/5125/84649/**5045**/51109/5959/25976/949/10404/1889/9249
receptor metabolism	receptor metabolic process	GO:0001503	0,000734	3579/284/1759/6424/634/7436/9370/948/3688/7037/10203/255738/3685/8862/84896/83891/57007/857/7422/133/2530/**5045**/55788/928/9779/43/5663/22853/1889
multicellular organism metabolism	multicellular organism metabolic process	GO:0044236	8,03E-08	1295/1301/153/1281/1303/1293/1290/1277/3688/1289/9902/2149/1282/652/50509/1278/1284/10891/1490/5159/115209/2690/1513/90993/4327/4239/871/80781/51430/**5045**/9509/1306/25976/9697/1508/4324/1292
	regulation of transforming growth factor beta 1 production	GO:0032908	0,004902	10159/7057/4060/**5045**/55353
	multicellular organismal catabolic process	GO:0044243	1,03E-06	1295/1301/1281/1303/1293/1290/1277/3688/1289/9902/1282/50509/1278/1284/1513/4327/80781/**5045**/9509/1306/1508/4324/1292
extracellular matrix organization	extracellular matrix organization	GO:0030198	1,27E-31	9/1278/23213/3908/7057/1311/3685/6678/4060/9806/84695/1284/3655/1490/4851/2621/30001/83700/169611/3915/151887/1605/2199/351/538/1513/90993/4327/4239/9644/90102/871/649/4763/80781/7045/**5045**/3910/9509/3339/30008/56999/84168/176/4324/1292
	extracellular structure organization	GO:0043062	1,27E-31	57/1311/3685/6678/4060/9806/84695/1284/3655/1490/4851/2621/30001/83700/169611/3915/151887/1605/2199/351/538/1513/90993/4327/4239/9644/90102/871/649/4763/80781/7045/**5045**/3910/9509/3339/30008/56999/84168/176/4324/1292
cellular response to transforming growth factor beta stimulus	cellular response to transforming growth factor beta stimulus	GO:0071560	2,12E-06	1149/2661/65997/55553/80310/8516/2201/1281/1003/2263/8754/5139/1277/50848/2200/7071/2331/23592/6422/7049/4053/7046/1278/94031/7057/5796/1284/10891/283149/94/83891/57045/135228/4052/857/7123/2734/2530/6497/**5045**/6935/657/7048
	transmembrane receptor protein serine/threonine kinase signaling pathway	GO:0007178	3,25E-07	8754/50848/2200/7071/4038/2331/51496/23592/6422/7049/652/4053/7046/1278/23213/6711/91851/94031/7057/3624/5796/283149/94/10140/4851/83891/151742/57045/1954/135228/4052/857/659/4012/2734/2530/6497/**5045**/92/6935/4756/657/7048
protein maturation	protein maturation	GO:0051604	0,00042	7057/79147/4311/25825/10699/2621/30001/2033/5270/115209/9986/4179/444/7123/143458/2734/10269/871/1191/5125/716/85409/**5045**/10098/7466/9509/841/5627/9528/84236/5621/81619/23385/5663/2934/1889
	protein processing	GO:0016485	0,001089	729/102/1370/1362/1368/255738/1363/10159/8473/3075/5727/2/2619/120892/7057/79147/4311/25825/10699/2621/5270/115209/9986/4179/444/7123/143458/2734/10269/1191/5125/716/85409/**5045**/9509/841/5627/9528/84236/5621/23385/5663/2934/1889
	membrane protein ectodomain proteolysis	GO:0006509	5,19E-05	150/102/8754/51752/5774/25825/152503/1605/9860/84888/**5045**/23621/23385/5663

Note: This board shows: the GO terms that includes FURIN (enriched in the set of genes down-regulated by irisin) and their description, the adjusted q-value, the number of geneIDs to each term_description is showed, highlighted FURIN (ID5045).

Table 2
TreeMap analysis for the terms involves *ADAM10* and other genes Down regulated by Irisin. The grouped terms and the enriched genes are represented for each main term found after the TreeMap cluster analysis; 12 terms are grouped into 3 main terms (Representative_term).

Representative_term	Term_description	Term_ID	qvalue	geneID
negative regulation of cell adhesion	negative regulation of celladhesion	GO:0007162	1,40E-05	1600/3371/58494/284/**102**/9750/634/1366/3077/9370/1277/9037/1545/347/2192/8763/124872/5140/652/27242/340485/7057/5592/6695/84695/94/4851/57045/8322/2064/5270/81/90102/4763/91663/7045/6591/928/1739/5621/23240/114897/8324/8573
	integrin-mediated signaling pathway	GO:0007229	3,89E-05	3691/3696/3683/8516/3672/**102**/634/1281/3680/8754/7070/3688/2185/22801/3685/84695/3655/1490/9510/2013/8829/2530/667
	regulation of chemotaxis	GO:0050920	0,016453	5919/6405/1991/80310/5156/**102**/9750/25960/9037/2185/2277/91584/7057/10371/196527/4851/2621/83700/5159/1954/8829/659/6091/7422/55558/2260
	Notch signaling pathway	GO:0007219	0,034411	79633/1004/**102**/4853/55534/3572/375033/6400/182/4851/2033/51378/351/4854/4179/57534/6591/63917/10098/81619/23385/5663/7048
	positive regulation of cellular component movement	GO:0051272	1,74E-06	10630/6546/4920/5919/1991/80310/5293/5156/150/284/10512/338879/**102**/388591/9750/1366/8754/3037/2549/25960/1277/9037/3479/7070/2185/2149/2277/652/7046/81704/7057/3685/8613/6678/10371/9806/3480/2252/3655/196527/4851/2621/5159/1954/51378/57007/8829/351/538/80031/5168/23224/659/57142/80005/81/596/958/25777/7422/4638/80781/91663/6591/2260/8828/4162/80149/949/8895/1956/388/7048
cell growth	cell growth	GO:0016049	7,04E-07	54437/79625/552/8839/10235/2661/6405/**102**/51232/634/2012/9201/347252/9037/148/3486/10516/54413/3479/8470/3688/2185/6586/3488/2487/6422/4137/91584/51196/7046/94031/5167/6695/3624/3685/10371/6098/131566/214/1490/94/4897/6595/5802/1954/1382/10505/8829/10479/2064/2697/5270/5538/351/9706/84162/659/57142/596/7422/56994/1535/27346/9538/55558/3339/56920/7414/8828/79602/367/1956/3490/7048/7216/1499
	extracellular structure organization	GO:0043062	1,27E-31	64094/3691/3696/79625/10630/1805/3371/3683/2824/1295/1301/1991/4148/5156/8516/58494/2201/1634/3672/23768/**102**/83716/1842/1281/4811/1303/1293/9507/3680/3037/1290/1277/5212/5033/50848/8434/1462/1545/8076/2200/2006/11096/10516/3688/4017/22801/1289/2192/7412/8038/2331/4015/1282/2/7837/7046/51144/50509/1278/23213/3908/7057/1311/3685/6678/4060/9806/84695/1284/3655/1490/4851/2621/30001/83700/169611/3915/151887/1605/2199/351/538/1513/90993/4327/4239/9644/90102/871/649/4763/80781/7045/5045/3910/9509/3339/30008/56999/84168/176/4324/1292
	extracellular matrix organization	GO:0030198	1,27E-31	64094/3691/3696/79625/10630/1805/3371/3683/2824/1295/1301/1991/4148/5156/8516/58494/2201/1634/3672/23768/**102**/83716/1842/1281/4811/1303/1293/9507/3680/3037/1290/1277/5212/5033/50848/8434/1462/1545/8076/2200/2006/11096/10516/3688/4017/22801/1289/2192/7412/8038/2331/4015/1282/2/7837/7046/51144/50509/1278/23213/3908/7057/1311/3685/6678/4060/9806/84695/1284/3655/1490/4851/2621/30001/83700/169611/3915/151887/1605/2199/351/538/1513/90993/4327/4239/9644/90102/871/649/4763/80781/7045/5045/3910/9509/3339/30008/56999/84168/176/4324/1292
protein maturation	protein maturation	GO:0051604	0,00042	150/**102**/8754/51752/5774/25825/51360/152503/1605/9860/84888/5045/23621/84236/121665/23385/5663
	protein processing	GO:0016485	0,001089	729/**102**/1370/1362/1368/255738/1363/10159/8473/3075/5727/2/2619/120892/7057/79147/4311/25825/10699/2621/5270/115209/9986/4179/444/7123/143458/2734/10269/1191/5125/716/85409/5045/9509/841/5627/9528/84236/5621/23385/5663/2934/1889
	membrane protein ectodomain proteolysis	GO:0006509	5,19E-05	150/**102**/8754/51752/5774/25825/152503/1605/9860/84888/5045/23621/23385/5663
	membrane protein proteolysis	GO:0033619	0,0000379	150/**102**/8754/51752/5774/25825/51360/152503/1605/9860/84888/5045/23621/84236/121665/23385/5663

Note: This board shows: the GO terms that includes *ADAM10* (enriched in the set of genes down-regulated by irisin) and their description, the adjusted q-value, the number of geneIDs to each term_description is showed, highlighted *ADAM10* (ID102).

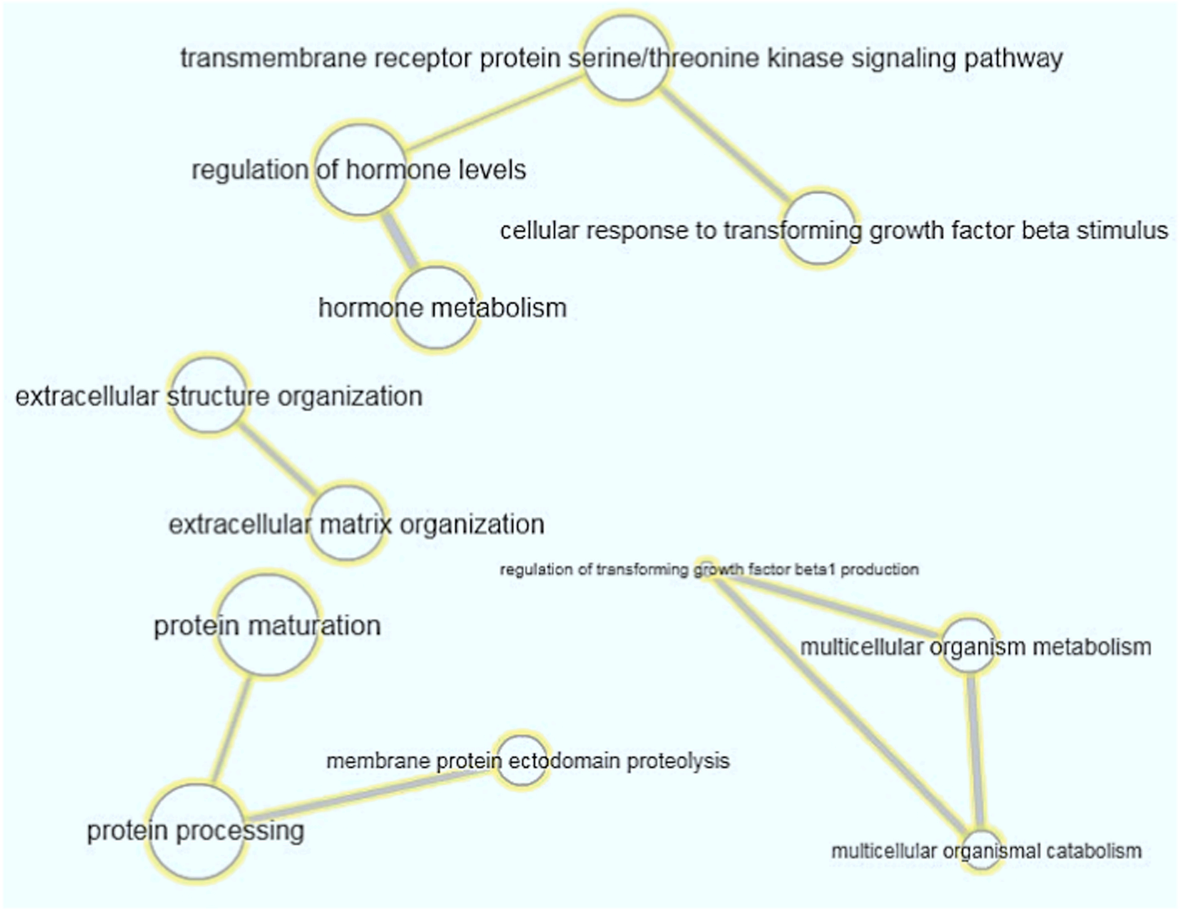

Fig. 1. Enriched GO terms, which involve down regulation of *FURIN* gene in the irisin group. Larger circles represent general terms and smaller circles represent specific terms; terms joined by lines indicate a relationship between them; 14 terms are represented in the REVIGO analysis.

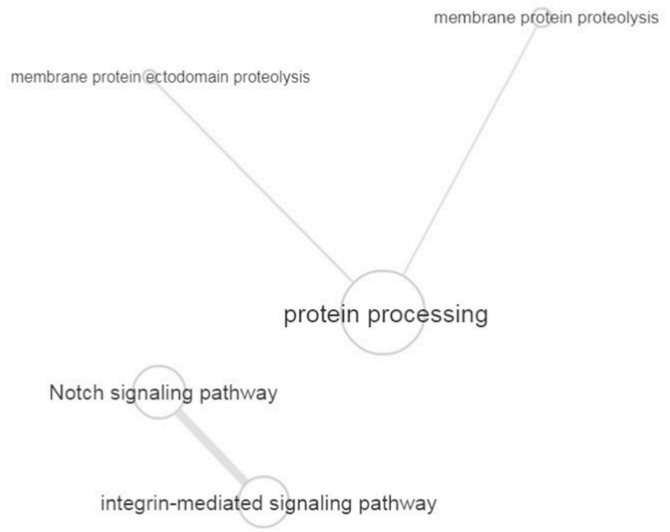

Fig. 2. Enriched GO terms, which involve down regulation of *ADAM10* gene in the irisin group. Larger circles represent general terms and smaller circles represent specific terms; terms joined by lines indicate a relationship between them; 12 terms are represented in the REVIGO analysis.
TRIB3 was elevated by Irisin.

genes were decreased in the presence of irisin; *HDAC2* was elevated, and *HAT1* and *RAB1A* expression were not altered by irisin action (Fig. 3).

4. Discussion

Obesity or overweight leads to reduced lung function, contributing to the reduction in lung volumes, especially the functional residual capacity in obese individuals compared with healthy individuals (Melo et al., 2014; Salome et al., 2010; Thyagarajan et al., 2008). *ACE2* is widely expressed in adipocytes and enriched in adipocytes of individuals with obesity and type II diabetes; there are *ACE2* regulator genes in the human lung, and the protein is used by SARS-CoV-2 to infect alveolar epithelial cells, resulting in the development of COVID-19 (Kruglikov and Scherer, 2020). These observations should be taken into consideration when an obese patient presents SARS-CoV-2 infection (Dietz and Santos-Burgoa, 2020). In our transcriptome data from human subcutaneous adipocytes, there was no detection of *ACE2* expression, what is probably due to the fact that the subcutaneous adipocytes used are from normal individuals, and the expression of *ACE2* is low to the point that it might be undetected by the parameters determined in our study for the RNA-seq technique; however the cell line showed the expression of multiple genes related to severe COVID- 19, such as *FURIN, ADAM10, TLR3, KDM5B, SIRT1 and TRIB3*.

Our data showed that irisin treatment decreased the levels of *FURIN, ADAM10, TLR3, KDM5B* and *SIRT1* mRNA expression, and increased the levels of *TRIB3* transcript by 3-fold. These results come together with the beneficial results that irisin has shown in a recent study published by our group (de Oliveira et al., 2020), in which irisin improved uncoupling protein 1 (*UCP1*) production, reduced lipid profile and oxidative stress, while not altering leptin, adiponectin, peroxisome proliferator-activated receptor gamma (*PPARγ*) and FNDC5 levels. According to anti-obesity effects of irisin on human a recent

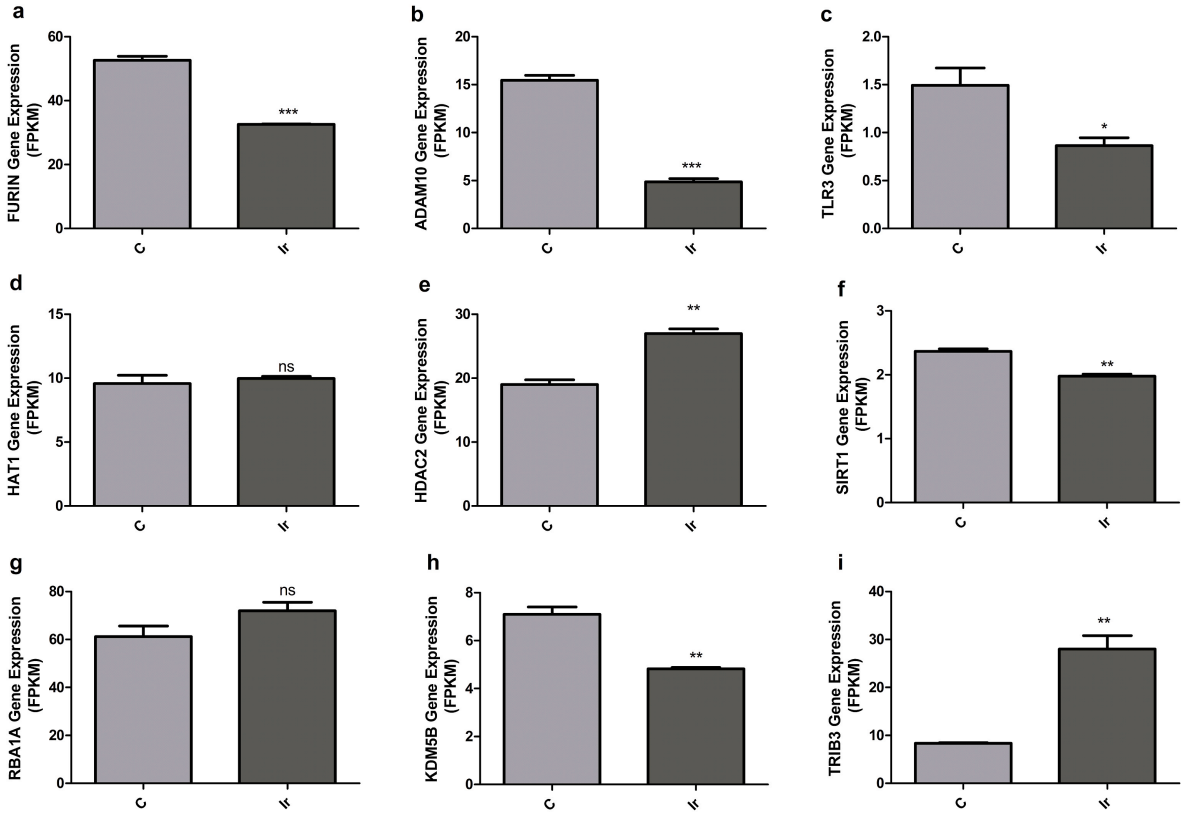

Fig. 3. Irisin effect on *FURIN, ADAM10, TLR3, HAT1, HDAC2, SIRT1, RAB1A, KDM5B* and *TRIB3,* in subcutaneous adipocytes: Human subcutaneous adipocytes were treated with 20 nm irisin for 24h, after this period the RNA extraction, preparation and sequencing was performed. Bioinformatics analysis was performed using Kallisto software. a) *FURIN*, b) *ADAM10*, c) *TLR3*, f) *SIRT1* and h) *KDM5B* were decreased by irisin e) HDAC2 and i) *TRIB3* were elevated by irisin. The values expressed in fragments per kilobase of exon model per million reads mapped (FPKM), from RNA-seq, were used to compare irisin group (Ir) with control (C); using Student's t-test to compare results, after performing the Kolmogorov Smirnov normality test. Data are expressed as mean ± standard deviation. The significance level was set at 5%. $^{**}P < 0.001$, $^{***}P < 0.0001$ n = 3, ns = non significant.

study has shown that irisin also inhibits lipogenesis and enhances fat browning in human adipocytes obtained from visceral adipose tissue, a fat depot with worst metabolic profile (Frühbeck et al., 2020). Although a considerable number of studies have so far demonstrated a beneficial effect of irisin on obesity (Boström et al., 2012; Frühbeck et al., 2020; de Oliveira et al., 2020; Panati et al., 2016), it must be considered that the major reason behind this outcome is its the ability of irisin to induce adipocyte browning, in a process known as irisin's canonical function, which largely depends on the experimental conditions or the distinct adipocyte subsets used (Gassenhuber et al., 2013; Lee et al., 2014; Silva et al., 2014; Kristóf et al., 2015; Klusóczki et al., 2019). In a research from 2015, Kristóf and colleagues (Kristóf et al., 2015) suggest that irisin is able to induce a “beige” program in differentiating human primary subcutaneous white adipocytes, and not effectively browning; the protocol used consisted of irisin administration during the whole differentiation procedure, or in the last four days of the differentiation, and the cell lineage employed was of preadipocytes. In addition, Lee and coworkers (Lee et al., 2014) showed that FNDC5 improved thermogenesis in subcutaneous but not omental adipocytes, and the magnitude of thermogenic activation was less evident compared to those observed in neck adipocytes. Therefore, despite the divergent results in regard to irisin's effect and FNDC5's participation in different fat deposits, previous results by Oliveira and colleagues (de Oliveira et al., 2020) using human subcutaneous adipocytes incubated for 24 h with 20 nM irisin indicated that irisin may be used to prevent obesity or maintain weight due to its impact on the lipid profile without altering adipokine levels; in this present article, it was demonstrated what irisin might, in addition, lead to reduction of *FURIN, ADAM10, TLR3, KDM5B* and *SIRT1* along with increase in *TRIB3* mRNA expression.

FURIN cleaves SARS-CoV-2 spike (S) glycoprotein, the key protein used to infect mammal cells (Walls et al., 2020); *ADAM10* is correlated with *ACE2* cleavage regulation in human airway epithelia (Jia et al., 2009); *TLR3* plays an important role in the innate response to SARS-CoV or MERS-CoV infection and regulates *ACE2* cleavage (Totura et al., 2015); *KDM5B* regulates positively *ACE2* (Wu et al., 2018); in more than 57% addressed studies, researchers found that *SIRT1* was upregulated in the lung of patients with severe COVID-19 comorbidities; all these genes favor the viral infection and can be potential targets for preventing SARS-CoV-2 spread (Pinto et al., 2020). On the other hand, *TRIB3* gene has been linked to fatty acid synthesis control and insulin resistance, in addition to regulating plasma levels of triglycerides and HDL cholesterol in humans (Angyal and Kiss-Toth, 2012) and was previously reported to decrease virus infection and replication; so *TRIB3* can be considered a therapeutic target for COVID-19 (Moraes et al., 2020).

Irisin is found in human blood at concentrations of 3–5 ng/ml; circulates at ~3.6 ng/ml in sedentary individuals; this level is increased to ~4.3 ng/ml in individuals undergoing aerobic interval training (Jedrychowski et al., 2015). The involvement of irisin in viral infection is poorly understood; however, a cross-sectional study of patients with HIV demonstrated that irisin levels correlated negatively with body fat and positively with fat-free mass and strength parameters (Trombeta et al., 2017). In another scenario, researchers verified that *FNDC5* overexpression or irisin supplementation could preserve mitochondrial function and attenuate oxidative damage as well as cell apoptosis (Ling et al., 2018; Zhang et al., 2014).

Irisin is known for stimulating markers of the white adipose tissue browning process contributing to energy metabolism, tissue

regeneration, cell proliferation and also in decreasing insulin resistance, helping to transport glucose into the muscles and consequently, lowering blood glucose (Ling et al., 2018). However, its effects on the hormonal regulation and metabolic process are not completely understood. We demonstrated that irisin reduced *FURIN*, which participates along with other genes, also downregulated by irisin, in the enrichment of 14 terms in GO. The terms include protein maturation, cellular response to transforming growth factor-beta stimulus, extracellular matrix organization, multicellular organism metabolism, regulation of lipase activity, and receptor metabolism; the interaction between these terms can be seen in Fig. 1. *FURIN* is one of the proteases responsible for the proteolytic cleavage of avian influenza virus activating the haemagglutinin (Stieneke-Gröber et al., 1992), and for the cleavage of human immunodeficiency virus (HIV) envelope polyprotein precursor gp160 to gp120 and gp41 prior to viral assembly (Hallenberger et al., 1992). Therefore, our results proving that irisin downregulates *FURIN,* which presents a proved impact on SARS-CoV-2 cleavage to human cells, may indicate that the treatment with irisin, through *FURIN* reduction, could consequently result in impaired SARS-CoV-2 infection. In addition, we observed that the reduction of *ADAM10,* along with other genes, after irisin enrichment resulted in 12 terms in GO, represented by protein maturation, negative regulation of cell adhesion and cell growth, as described in table. 2 and in interactive graphic in Fig. 2. Since *ADAM10* can be a candidate enzyme responsible for the *FNDC5* cleavage (Yu et al., 2019), the reduction of *ADAM1*0 mRNA in our study can be explained by the administration of exogenous irisin. The reduction of *ADAM1*0 by irisin resulted in the enrichment of the biological process Notch signaling pathway; because this pathway is thought to play a major role during the differentiation and the activity of innate and adaptive immune cells (Vieceli Dalla Sega et al., 2019), it is possible that irisin treatment indirectly results in a more efficient immune system.

Moreover, our data showed that the diminished *ADAM10* and *FURIN* lead to the enrichment of common processes such as extracellular structure organization, protein maturation, protein processing membrane and protein ectodomain proteolysis. Anders et al. (2001) and Hwang et al. (2006) described that the activity of *ADAM10* is also controlled by furin protein, which could be in line with our findings since treatment with irisin decreased FURIN and ADAM10 mRNA. The reduction of both genes could be beneficial for cell and extracellular processes.

Acute respiratory distress syndrome (ARDS) is the definition of the most severe form of acute lung injury (ALI). ARDS and ALI are severe clinical conditions that are associated with excessive inflammatory response, sepsis, impaired gas exchange, alveolar-capillary barrier disruption, and pulmonary edema (Rubenfeld et al., 2005; Wheeler and Bernard, 2007; Matthay et al., 2012; Imai et al., 2008; Yu et al., 2016). Therefore, patients with ALI/ARDS, among other symptoms, present decreased oxygenation capacity, bilateral lung ground-glass edge on chest radiographs, and an increase in breathing rate (Imai et al., 2008; Yu et al., 2016). A recent histopathological study demonstrated that irisin ameliorated the lung injury that was induced by lipopolysaccharides (LPS) in C57BL/6J mice and A549 cell (adenocarcinomic human alveolar basal epithelial cells), and suppressed the production of IL-1β, IL-6, MCP-1, TNF-α, and apoptosis in alveolar epithelial cells (Shao et al., 2017b). Although alveolar cells do not express irisin, previous studies using exogenous irisin indicated that these cells benefit from irisin in case the pulmonary tissue is damaged; the administration of irisin in mice subjected to ischemia demonstrated that inflammation in the lungs was reduced, there was evident tissue repair, and hypoxemia and proinflammatory cytokines were decreased. (Chen et al., 2017). The clinical characteristics of SARS-CoV-2 infection are varied and are similar to those described above by ARDS e ALI (Chen et al., 2020; Rubenfeld et al., 2005; Wheeler and Bernard, 2007; Matthay et al., 2012; Imai et al., 2008; Yu et al., 2016), a fact that reinforces testing irisin in cases of COVID-19. These data also support a promising potential for irisin in the battle against SARS-CoV-2 infection, and since literature in this domain is scarce, further researches are required.

The data presented by Pinto et al. (2020) and Moraes et al., 2020 (Moraes et al., 2020) offer gene targets for preventing severe COVID-19. Drugs that act on these gene targets, inhibiting *ADAM10, TLR3, HAT1, HDAC2, KDM5B, SIRT1, RAB1A* and *FURIN*, or stimulating *TRIB3*, should be explored. Our results from adipose tissue treatment with irisin showed a decrease in the expression of practically all targets mentioned by Pinto et al. (2020) and increased *TRIB3* mentioned by Moraes et al., 2020) (Moraes et al., 2020). Researches conducted in the lung tissue are required in order to validate if the same occurs in pulmonary spectrum.

In conclusion, irisin presented a very positive effect in the regulation of diverse genes related to the COVID-19 outcome in the adipose tissue, causing reduction of genes implicated in elevated viral infection, and increase in genes that block virus-cell cleavage; these results indicate that irisin treatment could end in a reduction of SARS-CoV-2 infection rate in human cells. The obesity is associated with an increased recruitment of macrophages in the adipose tissue (Weisberg et al., 2003), and irisin modulates macrophage activity by reducing reactive oxygen species (ROS) overproduction and increasing the expression of anti-oxidative stress factors (Mazur-Bialy, 2017; Mazur-Bialy et al., 2018); thus, the beneficial effects of irisin not only depend on the positive effect on the regulation of diverse genes related to the COVID-19 outcome in the adipose tissue, but also to its anti-inflammatory properties related to the targeting of macrophages. These results might be translatable for other tissues and systems, considering the genes targeted are expressed in several body cell types. Since the respiratory system is the most considerably affected in COVID-19, the effect of irisin in the lung tissue and the resulting gene expression must be tested. Therefore, we expect with our data, while preliminary and speculative, to stimulate further investigations relating to irisin and gene expression in other tissues than the adipose. This might evoke a COVID-19 therapeutic strategy to stimulate *TRIB3* expression and decrease *ACE2* regulatory genes, possibly aiding patients with both mild outcomes as well as the ones suffering from severe COVID-19.

Declaration of competing interest

The authors have nothing to disclose.

CRediT authorship contribution statement

Miriane de Oliveira: Conceptualization, Methodology, Data curation, Writing - original draft, Writing - review & editing. **Maria Teresa De Sibio:** Visualization, Investigation. **Lucas Solla Mathias:** Visualization, Investigation. **Bruna Moretto Rodrigues:** Visualization, Investigation. **Marna Eliana Sakalem:** Visualization, Investigation. **Célia Regina Nogueira:** Supervision.

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References

Anders, A., Gilbert, S., Garten, W., Postina, R., Fahrenholz, F., 2001. Regulation of the alpha-secretase ADAM10 by its prodomain and proprotein convertases. Faseb. J. 15

(10), 1837–1839. https://doi.org/10.1096/fj.01-0007fje.
Angyal, A., Kiss-Toth, E., 2012. The tribbles gene family and lipoprotein metabolism. Curr. Opin. Lipidol. 23 (2), 122–126. https://doi.org/10.1097/MOL.0b013e3283508c3b.
Boström, P., Wu, J., Jedrychowski, M.P., et al., 2012. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 481 (7382), 463–468. https://doi.org/10.1038/nature10777. Published 2012 Jan 11.
Chen, K., Xu, Z., Liu, Y., et al., 2017. Irisin protects mitochondria function during pulmonary ischemia/reperfusion injury. Sci. Transl. Med. 9 (418), eaao6298.
Chen, N., Zhou, M., Dong, X., et al., 2020. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 395 (10223), 507–513. https://doi.org/10.1016/S0140-6736(20)30211-7.
GBD 2015 eastern mediterranean region obesity collaborators. Burden of obesity in the eastern mediterranean region: findings from the global burden of disease 2015 study. Int. J. Publ. Health 63 (Suppl. 1), 165–176. https://doi.org/10.1007/s00038-017-1002-5.
de Oliveira, M., Mathias, L.S., Rodrigues, B.M., et al., 2020. The roles of triiodothyronine and irisin in improving the lipid profile and directing the browning of human adipose subcutaneous cells. Mol. Cell. Endocrinol. 506, 110744. https://doi.org/10.1016/j.mce.2020.110744.
Dietz, W., Santos-Burgoa, C., 2020. Obesity and its implications for COVID-19 mortality. Obesity. https://doi.org/10.1002/oby.22818. 10.1002/oby.22818.
Frühbeck, G., Fernández-Quintana, B., Paniagua, M., et al., 2020. FNDC4, a novel adipokine that reduces lipogenesis and promotes fat browning in human visceral adipocytes [published online ahead of print, 2020. Metabolism 108, 154261. https://doi.org/10.1016/j.metabol.2020.154261.
Gassenhuber, H., et al., 2013. Evidence against a beneficial effect of irisin in humans. PloS One 8 (9), e73680. https://doi.org/10.1371/journal.pone.0073680.
GBD 2015 Obesity Collaborators, Afshin, A., Forouzanfar, M.H., et al., 2017. Health effects of overweight and obesity in 195 countries over 25 years. N. Engl. J. Med. 377 (1), 13–27. https://doi.org/10.1056/NEJMoa1614362.
Gheblawi, M., Wang, K., Viveiros, A., et al., 2020. Angiotensin converting enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system. Circ. Res. https://doi.org/10.1161/CIRCRESAHA.120.317015. [published online ahead of print, 2020 Apr 8], 10.1161/CIRCRESAHA.120.317015.
Hallenberger, S., Bosch, V., Angliker, H., Shaw, E., Klenk, H.D., Garten, W., 1992. Inhibition of furin-mediated cleavage activation of HIV-1 glycoprotein gp160. Nature 360 (6402), 358–361. https://doi.org/10.1038/360358a0.
Hwang, E.M., Kim, S.K., Sohn, J.H., et al., 2006. Furin is an endogenous regulator of alpha-secretase associated APP processing. Biochem. Biophys. Res. Commun. 349 (2), 654–659. https://doi.org/10.1016/j.bbrc.2006.08.077.
Imai, Y., Kuba, K., Neely, G.G., et al., 2008. Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. Cell 133 (2), 235–249. https://doi.org/10.1016/j.cell.2008.02.043.
Jedrychowski, M.P., Wrann, C.D., Paulo, J.A., et al., 2015. Detection and quantitation of circulating human irisin by tandem mass spectrometry. Cell Metabol. 22 (4), 734–740. https://doi.org/10.1016/j.cmet.2015.08.001.
Jia, H.P., Look, D.C., Tan, P., et al., 2009. Ectodomain shedding of angiotensin converting enzyme 2 in human airway epithelia. Am. J. Physiol. Lung Cell Mol. Physiol. 297 (1), L84–L96. https://doi.org/10.1152/ajplung.00071.2009.
Klusóczki, Á., Veréb, Z., Vámos, A., et al., 2019. Differentiating SGBS adipocytes respond to PPARγ stimulation, irisin and BMP7 by functional browning and beige characteristics. Sci. Rep. 9, 5823. https://doi.org/10.1038/s41598-019-42256-0.
Kristóf, E., Doan-Xuan, Q.M., Bai, P., Bacso, Z., Fésüs, L., 2015. Laser-scanning cytometry can quantify human adipocyte browning and proves effectiveness of irisin. Sci. Rep. 5, 12540. https://doi.org/10.1038/srep12540.
Kruglikov, I.L., Scherer, P.E., 2020. The role of adipocytes and adipocyte-like cells in the severity of COVID-19 infections. Obesity. https://doi.org/10.1002/oby.22856. 10.1002/oby.22856.
Lee, P., Linderman, J.D., Smith, S., et al., 2014. Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans. Cell Metabol. 19 (2), 302–309. https://doi.org/10.1016/j.cmet.2013.12.017.
Ling, L., Chen, D., Tong, Y., et al., 2018. Fibronectin type III domain containing 5 attenuates NLRP3 inflammasome activation and phenotypic transformation of adventitial fibroblasts in spontaneously hypertensive rats. J. Hypertens. 36 (5), 1104–1114. https://doi.org/10.1097/HJH.0000000000001654.
Madissoon, E., Wilbrey-Clark, A., Miragaia, R.J., Saeb-Parsy, K., et al., 2019. Lung, spleen and oesophagus tissue remains stable for scRNAseq in cold preservation. bioRxiv 741405. https://doi.org/10.1101/741405.
Matthay, M.A., Ware, L.B., Zimmerman, G.A., 2012. The acute respiratory distress syndrome. J. Clin. Invest. 122 (8), 2731–2740. https://doi.org/10.1172/JCI60331.
Mazur-Bialy, A.I., 2017. Irisin acts as a regulator of macrophages host defense. Life Sci. 176, 21–25. https://doi.org/10.1016/j.lfs.2017.03.011.
Mazur-Bialy, A.I., Kozlowska, K., Pochec, E., Bilski, J., Brzozowski, T., 2018. Myokine irisin-induced protection against oxidative stress in vitro. Involvement of heme oxygenase-1 and antioxidazing enzymes superoxide dismutase-2 and glutathione peroxidase. J. Physiol. Pharmacol. 69 (1), 117-125. https://doi.org/10.26402/jpp.2018.1.13.
Melo, L.C., Silva, M.A., Calles, A.C., 2014. Obesity and lung function: a systematic review. Einstein (Sao Paulo). 12 (1), 120–125. https://doi.org/10.1590/s1679-45082014rw2691.
Mokdad, A.H., Forouzanfar, M.H., Daoud, F., et al., 2016. Health in times of uncertainty in the eastern Mediterranean region, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet Glob Health 4 (10). https://doi.org/10.1016/S2214-109X(16)30168-1. e704–e713.
Moraes, D., Paiva, B.V.B., Cury, S.S., Araujo Jr., J.P., Mori, M.A.S., Carvalho, R.F., 2020. Prediction of SARS-CoV interaction with host proteins during lung aging reveals a potential role for TRIB3 in COVID-19. bioRxiv. https://doi.org/10.1101/2020.04.07.030767. 04.07.030767.
Moreno-Navarrete, J.M., Ortega, F., Serrano, M., et al., 2013. Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. J. Clin. Endocrinol. Metab. 98 (4), E769–E778. https://doi.org/10.1210/jc.2012-2749.
Panati, K., Suneetha, Y., Narala, V.R., 2016. Irisin/FNDC5–an updated review. Eur. Rev. Med. Pharmacol. Sci. 20, 689–697.
Perakakis, N., Triantafyllou, G.A., Fernández-Real, J.M., et al., 2017. Physiology and role of irisin in glucose homeostasis. Nat. Rev. Endocrinol. 13 (6), 324–337. https://doi.org/10.1038/nrendo.2016.221.
Ph, A., Abraham, G., Mathew, M., Sundaram, A., Ali, A.A.M., Vijayan, M., 2019. Quantification of subcutaneous and visceral fat in abdominal CT and the correlation with metabolic parameters. J. Assoc. Phys. India 67 (12), 38–40.
Pinto, B.G.G., Oliveira, A.E.R., Singh, Y., et al., 2020. ACE2 expression is increased in the lungs of patients with comorbidities associated with severe COVID-19. 2020. https://doi.org/10.1101/2020.03.21.20040261.
Rothe, C., Schunk, M., Sothmann, P., et al., 2020. Transmission of 2019-nCoV infection from an asymptomatic contact in Germany. N. Engl. J. Med. 382 (10), 970–971. https://doi.org/10.1056/NEJMc2001468.
Rubenfeld, G.D., Caldwell, E., Peabody, E., et al., 2005. Incidence and outcomes of acute lung injury. N. Engl. J. Med. 353 (16), 1685–1693. https://doi.org/10.1056/NEJMoa050333.
Salome, C.M., King, G.G., Berend, N., 2010. Physiology of obesity and effects on lung function. 1985. J. Appl. Physiol. 108 (1), 206–211. https://doi.org/10.1152/japplphysiol.00694.2009.
Shao, L., Meng, D., Yang, F., Song, H., Tang, D., 2017a. Irisin-mediated protective effect on LPS-induced acute lung injury via suppressing inflammation and apoptosis of alveolar epithelial cells. Biochem. Biophys. Res. Commun. 487 (2), 194–200. https://doi.org/10.1016/j.bbrc.2017.04.020.
Shao, L., Meng, D., Yang, F., Song, H., Tang, D., 2017b. Irisin-mediated protective effect on LPS-induced acute lung injury via suppressing inflammation and apoptosis of alveolar epithelial cells. Biochem. Biophys. Res. Commun. 487 (2), 194–200. https://doi.org/10.1016/j.bbrc.2017.04.020.
Silva, F.J., Holt, D.J., Vargas, V., et al., 2014. Metabolically active human brown adipose tissue derived stem cells. Stem Cell. 32 (2), 572–581. https://doi.org/10.1002/stem.1595.
Singhal, T., 2020. A review of coronavirus disease-2019 (COVID-19). Indian J. Pediatr. 87 (4), 281–286. https://doi.org/10.1007/s12098-020-03263-6.
Stadnytskyi, V., Bax, C.E., Bax, A., Anfinrud, P., 2020. The airborne lifetime of small speech droplets and their potential importance in SARS-CoV-2. Proc. Natl. Acad. Sci. U. S. A. 202006874. https://doi.org/10.1073/pnas.2006874117.
Stieneke-Gröber, A., Vey, M., Angliker, H., et al., 1992. Influenza virus hemagglutinin with multibasic cleavage site is activated by furin, a subtilisin-like endoprotease. EMBO J. 11 (7), 2407–2414.
Supek, F., Bošnjak, M., Škunca, N., Šmuc, T., 2011. REVIGO summarizes and visualizes long lists of gene ontology terms. PloS One 6 (7), e21800. https://doi.org/10.1371/journal.pone.0021800.
Thomas, G., 2002. Furin at the cutting edge: from protein traffic to embryogenesis and disease. Nat. Rev. Mol. Cell Biol. 3 (10), 753–766. https://doi.org/10.1038/nrm934.
Thyagarajan, B., Jacobs Jr., D.R., Apostol, G.G., et al., 2008. Longitudinal association of body mass index with lung function: the CARDIA study. Respir. Res. 9 (1), 31. https://doi.org/10.1186/1465-9921-9-31. Published 2008.
Totura, A.L., Whitmore, A., Agnihothram, S., et al., 2015. Toll-like receptor 3 signaling via TRIF contributes to a protective innate immune response to severe acute respiratory syndrome coronavirus infection. e00638-15. mBio 6 (3). https://doi.org/10.1128/mBio.00638-15. Published 2015 May 26.
Tran, S.C., Pham, T.M., Nguyen, L.N., Park, E.M., Lim, Y.S., Hwang, S.B., 2016. Nonstructural 3 protein of hepatitis C virus modulates the tribbles homolog 3/akt signaling pathway for persistent viral infection. J. Virol. 90 (16), 7231–7247. https://doi.org/10.1128/JVI.00326-16. Published 2016 Jul 27.
Trombeta, J.C.D.S., Prestes, J., Nascimento, D.D.C., et al., 2017. New insights into the effects of irisin levels in HIV-infected subjects: correlation with adiposity, fat-free mass, and strength parameters. Arch Endocrinol Metab 61 (4), 382–390. https://doi.org/10.1590/2359-3997000000270.
Venkata, C., Sampathkumar, P., Afessa, B., 2010. Hospitalized patients with 2009 H1N1 influenza infection: the Mayo Clinic experience. Mayo Clin. Proc. 85 (9), 798–805. https://doi.org/10.4065/mcp.2010.0166.
Vieceli Dalla Sega, F., Fortini, F., Aquila, G., Campo, G., Vaccarezza, M., Rizzo, P., 2019. Notch signaling regulates immune responses in atherosclerosis. Front. Immunol. 10, 1130. https://doi.org/10.3389/fimmu.2019.01130.
Walls, A.C., Park, Y.J., Tortorici, M.A., Wall, A., McGuire, A.T., Veesler, D., 2020. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 181 (2), 281–292. https://doi.org/10.1016/j.cell.2020.02.058. e6.
Wang, X.F., Yuan, J., Zheng, Y.J., et al., 2020. Zhonghua Er Ke Za Zhi 58, E008. https://doi.org/10.3760/cma.j.issn.0578-1310.2020.0008. 0.
Weisberg, S.P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R.L., Ferrante Jr., A.W., 2003. Obesity is associated with macrophage accumulation in adipose tissue. J. Clin. Invest. 112 (12), 1796–1808. https://doi.org/10.1172/JCI19246.
Wheeler, A.P., Bernard, G.R., 2007. Acute lung injury and the acute respiratory distress syndrome: a clinical review. Lancet 369 (9572), 1553–1564. https://doi.org/10.1016/S0140-6736(07)60604-7.
World Health Organization Situation reports. Available at: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200413-sitrep-84-covid-19.pdf?sfvrsn=44f511ab_2.

Wu, L., Cao, J., Cai, W.L., et al., 2018. KDM5 histone demethylases repress immune response via suppression of STING. PLoS Biol. 16 (8), e2006134. https://doi.org/10.1371/journal.pbio.2006134.

Yu, J., Wang, Y., Li, Z., et al., 2016. Effect of heme oxygenase-1 on mitofusin-1 protein in LPS-induced ALI/ARDS in rats. Sci. Rep. 6, 36530. https://doi.org/10.1038/srep36530. Published 2016 Nov 10.

Yu, Q., Kou, W., Xu, X., et al., 2019. FNDC5/Irisin inhibits pathological cardiac hypertrophy. Clin Sci (Lond). 133 (5), 611–627. https://doi.org/10.1042/CS20190016.

Zanini, F., Pu, S.Y., Bekerman, E., Einav, S., Quake, S.R., 2018. Single-cell transcriptional dynamics of flavivirus infection. Elife 7, e32942. https://doi.org/10.7554/eLife.32942. Published 2018 Feb 16.

Zhang, Y., Li, R., Meng, Y., et al., 2014. Irisin stimulates browning of white adipocytes through mitogen-activated protein kinase p38 MAP kinase and ERK MAP kinase signaling. Diabetes 63 (2), 514–525. https://doi.org/10.2337/db13-1106.

Zhou, P., Yang, X.L., Wang, X.G., et al., 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579 (7798), 270–273. https://doi.org/10.1038/s41586-020-2012-7.