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Molecular and Functional Characterization of Human SW 872 Adipocytes as a Model System for Testing Nutraceutical Products †

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Abstract: Background The availability of characterized human adipocyte cell models for in vitro studies is currently limited. They may be useful to better understand the role of dysfunctional adipocytes in the pathophysiology of cardio-metabolic diseases and to evaluate the metabolic effects of nutraceutical compounds. In this study, human liposarcoma SW 872 cells, both non-differentiated and differentiated for 7 days with 100 mM oleic acid, have been used as the model system to 1. characterize these cells, concerning metabolic, pro-inflammatory and morphologic features, and 2. begin to evaluate the "healthy" effects of some plant-derived nutraceutical compounds. Methods In SW 872 cells, we evaluated the accumulation of triglycerides, the glucose uptake, the pro-inflammatory cytokine release and the modulation of Akt protein phosphorylation (pAkt), highlighting the differences between differentiated and non-differentiated cells. Results Oleic acid-differentiated SW 872 cells have a higher triglyceride content ($p < 0.001$) than non-differentiated cells, a lower glucose uptake ($p < 0.001$), but a higher insulin response ($p < 0.05$), and a specific activation of the Akt pathway. This cell model has been then chosen for the preliminary evaluation of the effects of some phytochemical complexes on the modulation of these molecular parameters, observing, in some instances, promising effects on reduction of triglyceride content and pro-inflammatory cytokine release as well as on increased glucose uptake. Conclusions Our study suggests that the SW 872 cell model could be useful for studies regarding human adipocyte function and dysfunction and the effects of bioactive compounds on dysfunctional adipose tissue, which will be addressed in future research.

Keywords: SW 872 cells; adipocytes; differentiation; bioactive compounds; cardio-metabolic diseases

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1. Introduction

Dysfunctional adipose tissue could lead to a large spectrum of diseases such as obesity, metabolic syndrome, atherosclerosis, and can influence the molecular pathophysiology of cardio-metabolic diseases [1,2]. However, many pathogenetic mechanisms concerning metabolism and in particular adipose tissue are still unknown, and, for this reason, it is important to implement cellular models that can best represent human adipocytes and their biological and biochemical mechanisms. These models would allow us to better understand the development and causes of diseases related to adipose tissue dysfunction, such as obesity and diabetes, and to improve the diagnostic strategies for such diseases. Moreover, the availability of standardized and validated human adipocyte cell

models would also facilitate many investigations aimed at testing novel compounds, nutrients and plant-derived extracts with potential nutraceutical activity [3,4]. More specifically, in vitro models allow us to study the differentiation of adipocytes, and to understand the molecular and cellular events that lead to mature adipocytes, from preadipocytes which are often more similar to non-differentiated fibroblasts. Among the cell lines commonly proposed as adipocyte models there is the 3T3-L1 preadipocyte murine cell line, which has been widely used in several studies, but shows the disadvantage of a low replicative capacity and consequently limited potential for in vitro differentiation [5]. Among the available adipocyte models of human origin, the human liposarcoma cell line, SW 872, has recently been developed. It represents an interesting human adipocyte model, which has been differentiated to more mature adipocytes through different protocols.

It is important to recall that the presence of dysfunctional adipose tissue, and therefore adipocytes, represents the pathophysiological driver of metabolic syndrome and associated complications. In this regard, dysfunctional adipocytes may show high oxidative stress, chronic low-grade inflammation, high pro-inflammatory cytokine release, insulin resistance and triglyceride accumulation. Nonetheless, there is no pharmacological treatment that acts directly on the dysfunctional adipose tissue, and nutritional and nutraceutical approaches are still to be fully developed. In this paper, we have addressed some aspects related to the biomolecular characterization of SW 872 cells, concerning their metabolic and morphologic features, and started to validate this cell-based model as a potential tool to evaluate in more detail the effects of bioactive compounds with potential nutraceutical properties.

2. SW 872 Adipocyte Differentiation

The SW 872 cell line has an immature adipocyte phenotype, constitutively expressing certain genes involved in the metabolism of fatty acids such as lipoprotein lipase (LPL), the cholesterol ester transfer protein (CETP), CD36, peroxisome proliferator-activated receptors-α (PPAR-α), peroxisome proliferator-activated receptors-γ (PPAR-γ) and LDL receptor related protein 1 (LRP1) [6]. The differentiation of these cells to mature adipocytes can be done with oleic acid (OA), a PPAR-γ agonist, which in previous studies had shown to increase triglycerides within SW 872 cells [6–8]. OA stimulates the PPAR-γ transcription factor, which is crucial for the regulation of specific adipocyte genes and has a fundamental role in adipocyte differentiation. There are some studies that mention additional methods of differentiation of these cells to mature adipocyte-like cells, like by adding dexamethasone (DEX), insulin and 3-isobutyl-1-methylxanthine (IBMX) [9]. Furthermore, it has been confirmed that these cells are capable of triglyceride accumulation, like mature adipocytes, simply by the addition of 100 μM OA in the culture medium [4]. In this case, SW 872 cells are grown in DMEM-F12 medium containing HEPES buffer, supplemented with 10% FBS, 1% penicillin, and streptomycin (100 μg/mL). The cells are kept in culture in Petri dishes at 37 °C in an atmosphere containing 5% CO_2. When a confluence of 80–100% is reached, a 100 μM solution of OA is added to initiate cell differentiation [7,10]. Additionally, in order to accelerate the induction of the differentiation process in 3 days only, a higher concentration of OA has also been used (0.5 mmol/L [11] or 0.6 mol/L [12]). Nonetheless, differentiated cells do not express the uncoupling protein 1 (UCP-1) and there is no change in the number of mitochondria present in the cell [10]. Fatty acid accumulation has been evaluated by Oil-Red-O lipid staining, in order to see the impact of differentiation on triglyceride accumulation (Figure 1A). The triglyceride content in undifferentiated cells slightly increased, after 24 h, by 49.63%, while after 48 h the triglyceride content increase was 63.96%. In contrast, cells differentiated with OA showed an almost double increase only after 24 h, compared to untreated cells (Figure 1B). Despite the considerable variation in the accumulation of triglycerides between differentiated and undifferentiated cells, no clear morphological cellular changes were induced by the treatment with OA. Resveratrol (10 μM), used as a positive control on differentiated cells, led to a 30.4% reduction of triglyceride cell content, while metformin (1 μM) and

epigallocatechin (40 μM) showed no changes in SW 872 cells lipid content (data not shown). Among all differentiation options, the OA treatment seems the best and simplest method to acquire mature adipocyte-like cells.

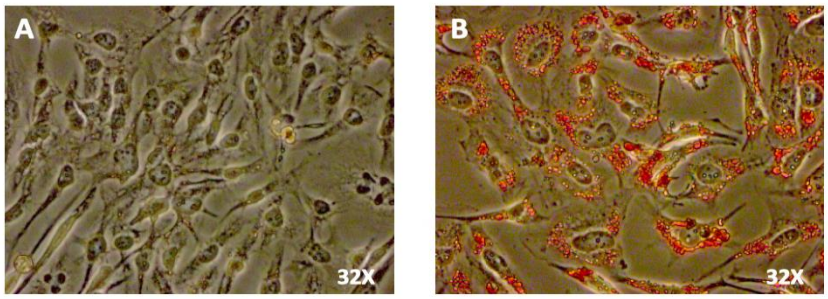

Figure 1. Analysis by Oil-Red-O staining of lipid content in (**A**) non-differentiated SW 872 cells and (**B**) SW 872 cells differentiated by treatment for 7 days with OA. Adapted from [3].

3. Glucometabolic Studies in SW 872 Cells

It is well known that dysfunctional adipocytes display a certain degree of insulin-resistance, with reduced glucose uptake, due to different molecular changes. Using SW 872 cells, glucose uptake was evaluated by observing the ability of these cells to internalize glucose (2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose; 2-NBDG) after treatment with OA [3]. Basal glucose uptake in SW 872 cells was reduced by 54.81% after treatment with OA, compared to non-differentiated cells (Figure 2A). The modulation of glucose uptake following insulin administration in differentiated and non-differentiated cells was evaluated as a function of time (15, 30 min and 1 h) and dose (10 nM, 100 nM and 1 μM). Although differentiated cells have lower glucose uptake than non-differentiated cells, it has been observed that these cells respond better to insulin. In particular, it has been shown that after an incubation of 60 min with 10 nM insulin, glucose uptake increased by 16.8%, and at the same time, 100 nM insulin led to an 18.8% increase in glucose absorption (Figure 2D). For all other conditions, insulin has not led to a significant increase in glucose uptake, as it did in non-differentiated SW 872 cells (Figure 2C). We then explored the role of the Akt pathway in these events. The phosphorylation of Akt protein is responsible for GLUT4 translocation on the cell membrane, its activation is induced upon insulin stimuli and therefore it has an impact on cell glucose uptake. We used the Western Blot technique to evaluate the presence and quantity of the Akt protein in SW 872 cell model, followed by the treatment of cells with 10 μM insulin [3]. In differentiated cells, Akt activation was undetectable at T0. Yet, since the Akt phosphorylation is an immediate event, after 5, 15 and 30 min pAkt amount increased, and it started leveling down after 1 h. In contrast, pAkt quantity was reduced in undifferentiated cells, even after insulin administration (Figure 2B). To summarize, SW 872 differentiated cells showed lower glucose uptake, which resembles that of dysfunctional adipocytes. Also, cells treated with OA, which is a PPAR-γ agonist, showed an increased response to insulin, as expected.

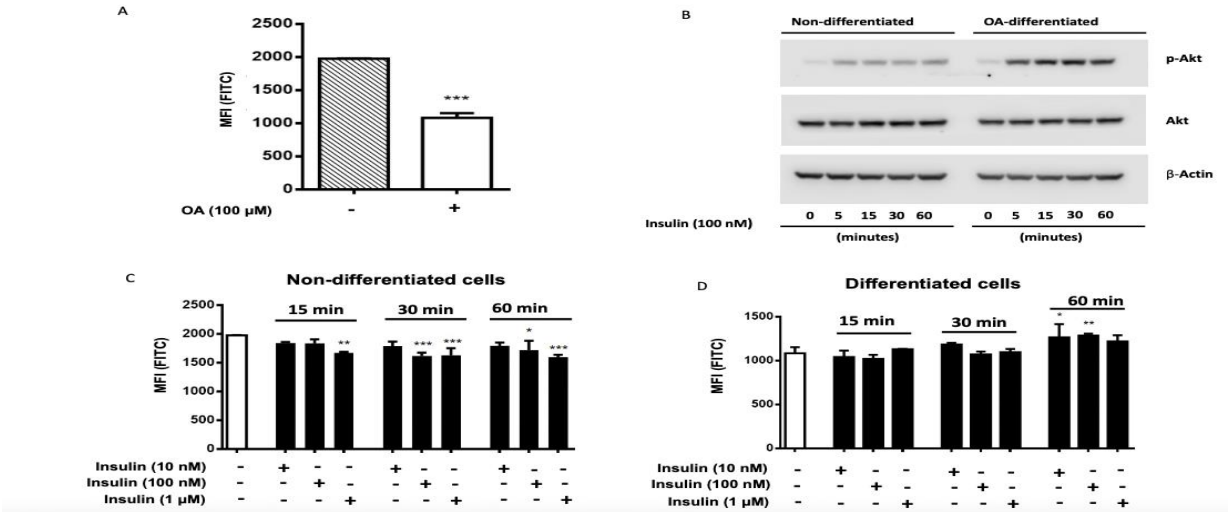

Figure 2. Modulation of glucose uptake in SW 872 cells. (**A**) Basal glucose absorption in non-differentiated and differentiated SW 872 cells. (**B**) Evaluation over time of Akt phosphorylation, following treatment with 100 nM insulin in non-differentiated and differentiated cells by Western Blot analysis. (**C**) Evaluation of the effect of insulin in time (15, 30 min and 1 h) and dose (10 nM, 100 nM and 1µM) response on glucose uptake in non-differentiated and (**D**) in differentiated cells. Glucose uptake was evaluated by analysis at FACS. An experiment (n = 3) representative of 3 separate experiments, each of which tripled is shown. The results are expressed as mean ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$ (one-way ANOVA multiple comparison). Adapted from [3].

4. Inflammation

Dysfunctional adipocytes release pro-inflammatory cytokines, like interleukin 6 (IL-6) and interleukin 8 (IL-8), leading to a high level of inflammation, which leads to worse health conditions. In our study, SW 872 cells showed a time-dependent modulation of the basal secretion of these pro-inflammatory cytokines, with an increase of IL-6 and a reduction of IL-8, after treatment with OA (Figure 3). Other studies show contradictory results on IL-6 and IL-8 release, depending on the PPAR-γ agonist and the adipocyte cell model used [13–15]. Considering these features, pro-inflammatory cytokine release and modulation must be further investigated, to validate and better understand which is the best dysfunctional adipocytes cell model.

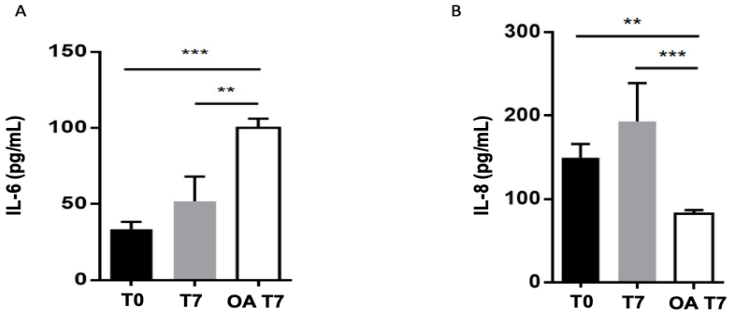

Figure 3. (**A**) Basal IL-6 content was determined at T0 and T7 (1 and 7 days after seeding, respectively) and at OA T7 (after 7-day differentiation with 100 µM OA). (**B**) Basal IL-8 content was determined at T0 and T7 (1 and 7 days after seeding, respectively) and at OA T7 (after 7-day differentiation with 100 µM OA). One experiment (n = 3) is shown as representative of 3 separate experiments, each in triplicate. Results are shown as mean ± SD. Data are expressed as pg/mL. ** $p < 0.01$, *** $p < 0.001$ (one-way ANOVA multiple comparison). Adapted from [3].

5. Modulation of Dysfunctional Aspects of SW 872 Cell Line by Plant Extracts

The SW 872 cell model was then utilized as a potential adipocyte model to test the effects of Cameroonian spice extracts to modify the above reported cellular mechanisms [3]. Interestingly, these plant extracts were found active in improving some of these functions in HepG2 human hepatocyte [16]. Table 1 reports the effects of a set of 11 Cameroonian on triglyceride content, glucose uptake stimulation, ROS production and cytokines release. Plant extracts were used only on differentiated cells, at different concentrations (1 μg/mL, 10 μg/mL and 20 μg/mL). The results showed that all plants reduced the triglyceride contents, while the other parameters were modulated by only some of them.

Table 1. Specific effects of the Cameroonian spice extracts tested on differentiated SW 872 cells. Adapted from [3].

	Triglyceride Reduction	Glucose Uptake Stimulation	ROS Production	IL−6 Reduction	IL−8 Reduction
Xylopia aethiopica	−14.5%		+55.8%		−21.1%
Xylopia parviflora	−13.8%		−50.5%		−36.8%
Scorodophloeus zenkeri	−18.5%				
Monodora myristica	−15.3%		−40%		−24.3%
Tetrapleura tetraptera	−13.8%	+40.8%	−27.4%	−29.7%	
Echinops giganteus	−11.3%		−43.6%	−29%	
Afrostyrax lepidophyllus	−16.5%		−24.6%		
Dichrostachys glomerata	−17.4%			−40%	
Aframomum melegueta	−13%	+41.7%		−43.1%	
Aframomum citratum	−16%				−58.6%
Zanthoxylum leprieurii	−13.4%	+56.6%			−32.7%

6. Conclusions and Discussion

The global prevalence of metabolic diseases such as obesity, type 2 diabetes mellitus (T2DM), metabolic syndrome and the consequent atherosclerotic cardiovascular diseases is increasing. In order to improve living conditions, the biochemical and molecular pathways of dysfunctional adipocytes have to be further investigated. To reach this aim, different dysfunctional adipocyte models are used. The SW 872 human liposarcoma model has been mainly used for in vitro studies in oncological studies, but there is also evidence that support its use for in vitro studies of dysfunctional adipose tissue and related diseases. Literature shows different methods to differentiate SW 872 cells to mature-like adipocytes, but to the best of our knowledge, there are no studies about fully characterizing these cells as a dysfunctional adipocyte model. It has been shown that this cell model can spontaneously differentiate into a mature adipocyte model, when placed in high density culture for 10 days [8]. This can be due both to the presence of triglycerides within the cell, and also to a number of other parameters. However, we decided to promote differentiation with 100 μM OA, as it induces a faster differentiation [3].

The intracellular lipids accumulation has been evaluated by ORO staining, showing a higher accumulation of triglycerides in differentiated cells than non-differentiated. This accumulation is similar to the one induced in adipocytes following activation of glitazone-mediated PPAR-γ, a side effect that frequently occurs in patients on glitazonic therapy for the treatment of T2DM [17].

Also, we evaluated the glucose uptake by cytofluorometric analysis, showing that non-differentiated SW 872 cells allow twice as much glucose input than differentiated cells, despite non-differentiated cells do not respond to insulin. In addition, the results obtained by Western Blotting analysis support this evidence; in fact, the phosphorylated Akt fraction in differentiated cells increases in a time dependent manner more markedly than in non-differentiated cells. Further study concerned the secretion of pro-inflammatory cytokines, showed an increase in IL-6 associated to differentiation; and it has also

been observed that secretion of IL-6 increases from T0 to T7 in non-differentiated cells. On the contrary, differentiated cells showed a lower release of IL-8 than non-differentiated cells. This can be due to the inhibition of IL-8 by PPAR-γ activation by OA, which is an agonist of the receptor [13]. Plant extracts of Cameroonian origin, used as nutritional spices and in traditional medicine, were found to modulate the glucometabolic and inflammatory aspects in SW 872 cells, suggesting that these cells could be used for the screening of functional compounds or extracts of natural origin (nutraceutical and plant extracts). Based on the highlighted characteristics, this model could be used in the future for the study of nutraceuticals or drugs for the treatment of cardiometabolic pathologies related to a dysfunction of adipose tissue.

References

1. Ross, R.; Neeland, I.J.; Yamashita, S.; Shai, I.; Seidell, J.; Magni, P.; Santos, R.D.; Arsenault, B.; Cuevas, A.; Hu, F.B.; et al. Waist circumference as a vital sign in clinical practice: A Consensus Statement from the IAS and ICCR Working Group on Visceral Obesity. *Nat. Rev. Endocrinol.* **2020**, *16*, 177–189. https://doi.org/10.1038/s41574-019-0310-7.
2. Neeland, I.J.; Ross, R.; Després, J.P.; Matsuzawa, Y.; Yamashita, S.; Shai, I.; Seidell, J.; Magni, P.; Santos, R.D.; Arsenault, B.; et al. Visceral and ectopic fat, atherosclerosis, and cardiometabolic disease: A position statement. *Lancet Diabetes Endocrinol.* **2019**, *7*, 715–725. https://doi.org/10.1016/S2213-8587(19)30084-1.
3. Atchan Nwakiban, A.P.; Passarelli, A.; Da Dalt, L.; Olivieri, C.; Demirci, T.N.; Piazza, S.; Sangiovanni, E.; Carpentier-Maguire, E.; Martinelli, G.; Shivashankara, S.T.; et al. Cameroonian Spice Extracts Modulate Molecular Mechanisms Relevant to Cardiometabolic Diseases in SW 872 Human Liposarcoma Cells. *Nutrients* **2021**, *13*, 4271. https://doi.org/10.3390/nu13124271.
4. Cicolari, S.; Dacrema, M.; Tsetegho Sokeng, A.J.; Xiao, J.; Atchan Nwakiban, A.P.; Di Giovanni, C.; Santarcangelo, C.; Magni, P.; Daglia, M. Hydromethanolic Extracts from Adansonia digitata L Edible Parts Positively Modulate Pathophysiological Mechanisms Related to the Metabolic Syndrome. *Molecules* **2020**, *25*, 2858. https://doi.org/10.3390/molecules25122858.
5. Fève, B. Adipogenesis: Cellular and molecular aspects. *Best Pract. Res. Clin. Endocrinol. Metab.* **2005**, *19*, 483–499. https://doi.org/10.1016/j.beem.2005.07.007.
6. Wassef, H.; Bernier, L.; Davignon, J.; Cohn, J.S. Synthesis and secretion of apoC-I and apoE during maturation of human SW872 liposarcoma cells. *J. Nutr.* **2004**, *134*, 2935–2941. https://doi.org/10.1093/jn/134.11.2935.
7. Carmel, J.F.; Tarnus, E.; Cohn, J.S.; Bourdon, E.; Davignon, J.; Bernier, L. High expression of apolipoprotein E impairs lipid storage and promotes cell proliferation in human adipocytes. *J. Cell. Biochem.* **2009**, *106*, 608–617. https://doi.org/10.1002/jcb.22037.
8. Stratford, E.W.; Castro, R.; Daffinrud, J.; Skårn, M.; Lauvrak, S.; Munthe, E.; Myklebost, O. Characterization of liposarcoma cell lines for preclinical and biological studies. *Sarcoma* **2012**, *2012*, 148614. https://doi.org/10.1155/2012/148614.
9. Fiorani, M.; De Matteis, R.; Canonico, B.; Blandino, G.; Mazzoli, A.; Montanari, M.; Guidarelli, A.; Cantoni, O. Temporal correlation of morphological and biochemical changes with the recruitment of different mechanisms of reactive oxygen species formation during human SW872 cell adipogenic differentiation. *BioFactors* **2021**, *47*, 837–851. https://doi.org/10.1002/biof.1769.
10. Guennoun, A.; Kazantzis, M.; Thomas, R.; Wabitsch, M.; Tews, D.; Seetharama Sastry, K.; Abdelkarim, M.; Zilberfarb, V.; Strosberg, A.D.; Chouchane, L. Comprehensive molecular characterization of human adipocytes reveals a transient brown phenotype. *J. Transl. Med.* **2015**, *13*, 135. https://doi.org/10.1186/s12967-015-0480-6.
11. He, Y.H.; He, Y.; Liao, X.L.; Niu, Y.C.; Wang, G.; Zhao, C.; Wang, L.; Tian, M.J.; Li, Y.; Sun, C.H. The calcium-sensing receptor promotes adipocyte differentiation and adipogenesis through PPARc pathway. *Mol. Cell. Biochem.* **2012**, *361*, 321–328.
12. Zhu, L.; Han, M.; Gao, Y.; Wang, H.; Dai, L.; Wen, Y.; Na, L. Curcumin triggers apoptosis via upregulation of Bax/Bcl-2 ratio and caspase activation in SW872 human adipocytes. *Mol. Med. Rep.* **2015**, *12*, 1151–1156. https://doi.org/10.3892/mmr.2015.3450.
13. Bruun, J.M.; Pedersen, S.B.; Richelsen, B. Interleukin-8 production in human adipose tissue. inhibitory effects of anti-diabetic compounds, the thiazolidinedione ciglitazone and the biguanide metformin. *Horm. Metab. Res.* **2000**, *32*, 537–541. https://doi.org/10.1055/s-2007-978682.
14. Azhar, S. Peroxisome proliferator-activated receptors, metabolic syndrome and cardiovascular disease. *Future Cardiol.* **2010**, *6*, 657–691. https://doi.org/10.2217/fca.10.86.
15. D'Esposito, V.; Passaretti, F.; Hammarstedt, A.; Liguoro, D.; Terracciano, D.; Molea, G.; Canta, L.; Miele, C.; Smith, U.; Beguinot, F.; et al. Adipocyte-released insulin-like growth factor-1 is regulated by glucose and fatty acids and controls breast cancer cell growth in vitro. *Diabetologia* **2012**, *55*, 2811–2822. https://doi.org/10.1007/s00125-012-2629-7.
16. Nwakiban, A.P.A.; Cicolari, S.; Piazza, S.; Gelmini, F.; Sangiovanni, E.; Martinelli, G.; Bossi, L.; Carpentier-Maguire, E.; Tchamgoue, A.D.; Agbor, G.; et al. Oxidative Stress Modulation by Cameroonian Spice Extracts in HepG2 Cells: Involvement of Nrf2 and Improvement of Glucose Uptake. *Metabolites* **2020**, *10*, 182. https://doi.org/10.3390/metabo10050182.
17. Cipolletta, D.; Feuerer, M.; Li, A.; Kamei, N.; Lee, J.; Shoelson, S.E.; Benoist, C.; Mathis, D. PPAR-γ is a major driver of the accumulation and phenotype of adipose tissue Treg cells. *Nature* **2012**, *486*, 549–553. https://doi.org/10.1038/nature11132.