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Whole transcriptome analysis by RNA-Seq, a state-of-the-art technique to uncover complex biological processes. The blackberry example.

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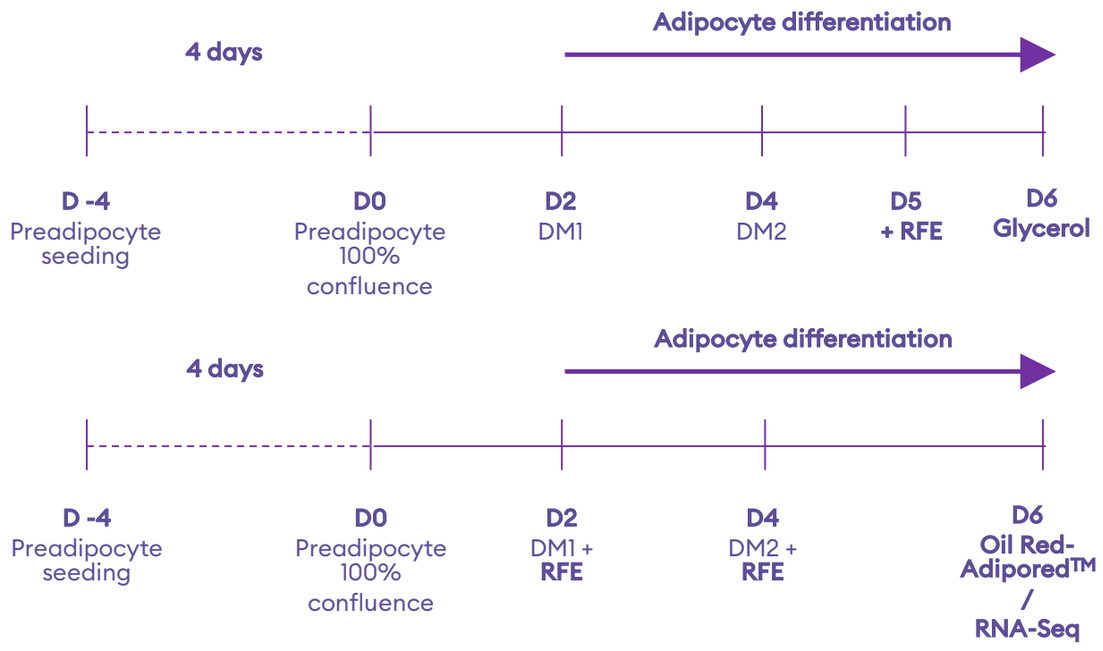
INTRODUCTION

The blackberry is linked to various health benefits, e.g. the prevention and treatment of metabolic syndrome, support of the digestive and immune system, prevention of inflammatory disorders, cardiovascular diseases and protective effects against gastrointestinal tract cancers [1]. The blackberry is also used in cosmetics, mainly due to its antioxidant and anti-inflammatory properties, making it suitable for skin antiaging and hair protection applications [2].

However, to our knowledge, currently there is no comprehensive description of the impact of the blackberry in fat accumulation, lipolysis or adipocyte differentiation. Nonetheless, some characteristic phytochemicals of the blackberry, e.g. cyanidin 3-glucoside, ellagitannins and flavonols, have been reported to exert activities on adipocytes or adipogenic-related processes [3–5]. Therefore, in the present work we sought to study the effect of a blackberry extract (Rubus fruticosus fruit extract, RFE) on adipogenesis and adipocyte metabolism by measuring lipid content in the adipocyte, by both Red Oil O and AdiporedTM staining, lipolysis by glycerol measurement, and by exploring gene expression patterns by RNA-Seq.

MATERIALS & METHODS

3T3-L1 cells were differentiated into adipocytes and stimulated with RFE for Glycerol or Oil Red-AdiporedTM and RNA-Seq analysis, following the protocols depicted below:



DM1: Differentiation Medium 1; DM2: Differentiation Medium 2

CONCLUSIONS

The present work shows, for the first time to our knowledge, that direct stimulation with a blackberry extract (Rubus fruticosus fruit extract, RFE) facilitates adipocyte differentiation in the 3T3-L1 cell model. RFE reduced lipolysis, quantified as glycerol measurement, and increased adipogenesis, as found by Red Oil O quantification and fluorescent AdipoRedTM staining. Furthermore, transcriptomic analysis by RNA-Seq revealed that RFE modulates the adipocyte differentiation transcriptomic fingerprint as a whole, including not only adipocyte metabolism, but also extracellular matrix remodeling. Our results support the notion that RFE may be of great interest for cosmetic treatments related with facial and body volumizing applications.

ACKNOWLEDGEMENTS

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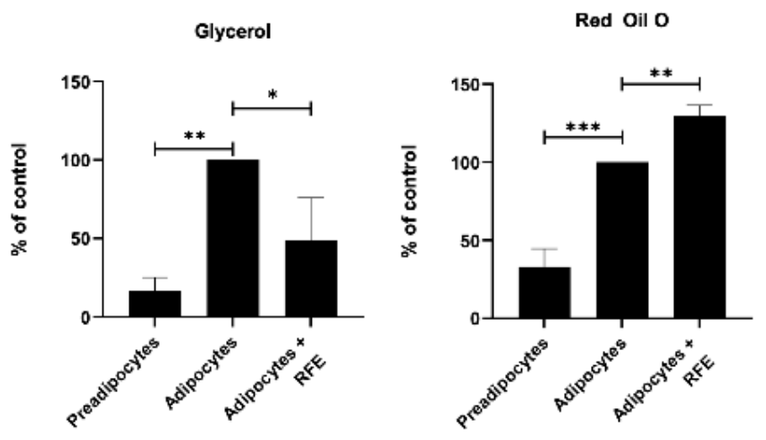
REFERENCES

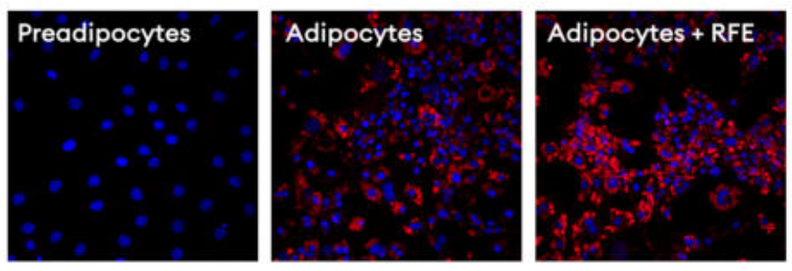
1. Kitrytė et al. “Consecutive high-pressure and enzyme assisted fractionation of blackberry (Rubus fruticosus L.) pomace into functional ingredients: Process optimization and product characterization,” Food Chem., vol. 312, no. July 2019, 2020.
2. Zia-Ul-Haq et al. “Rubus fruticosus L.: Constituents, biological activities and health related uses,” Molecules. 2014.
3. Matsukawa et al. “Cyanidin-3-glucoside derived from black soybeans ameliorate type 2 diabetes through the induction of differentiation of preadipocytes into smaller and insulin-sensitive adipocytes,” J. Nutr. Biochem., 20155.
4. Cisneros-Zevallos et al., “Ellagic acid and urolithins A and B differentially regulate fat accumulation and inflammation in 3T3-L1 adipocytes while not affecting adipogenesis and insulin sensitivity,” Int. J. Mol. Sci., 2020.
5. Fang et al. , “Kaempferol and quercetin isolated from Euonymus alatus improve glucose uptake of 3T3-L1 cells without adipogenesis activity,” Life Sci., 2008.

RESULTS & DISCUSSION

Lipolysis and adipogenesis



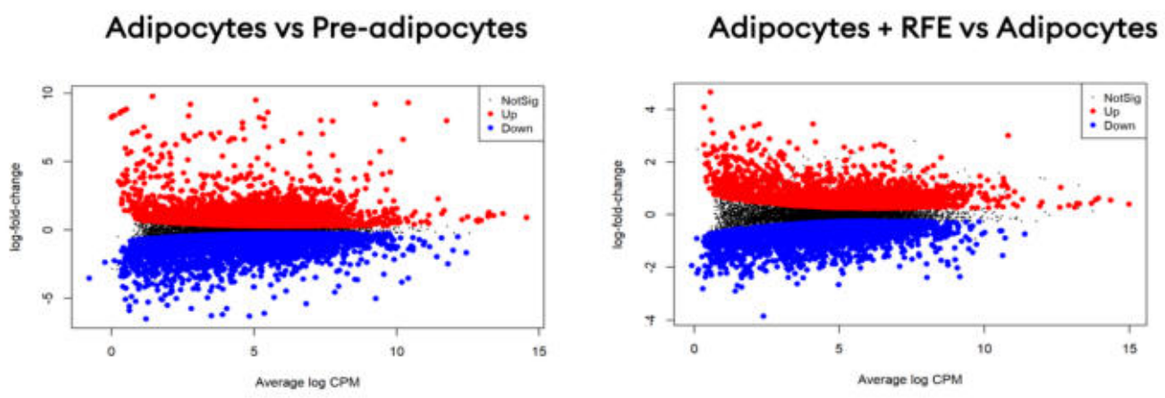
RFE promotes inhibition of lipolysis, measured as reduction of glycerol levels, and facilitates adipogenesis, as shown by increased Red Oil Staining.



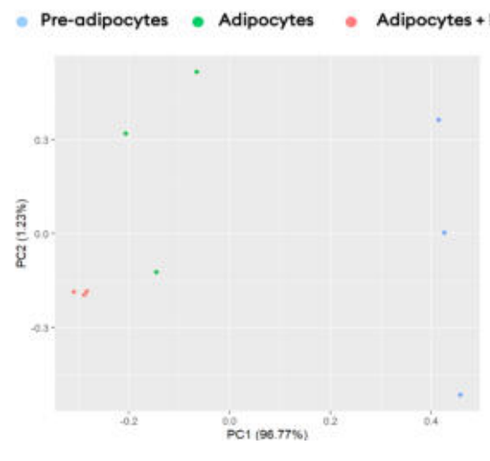
Hoechst AdipoRed™

Confocal images of lipid staining by AdipoRed™ also shows increased adipogenesis by RFE.

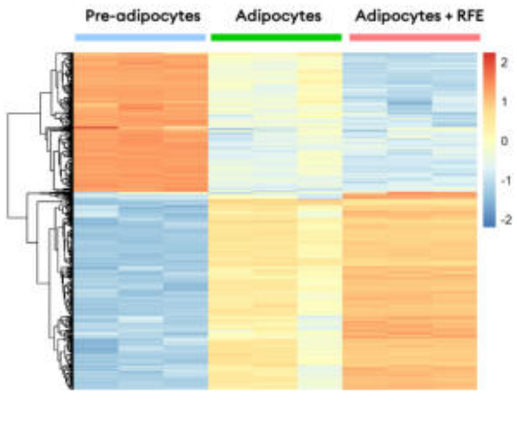
Transcriptome analysis by RNA-Seq



Adipocyte differentiation induces significant changes in 6.964 genes, while RFE significantly induced the change of 4.904 genes.



PCA analysis shows a clear separation between preadipocytes and differentiated adipocytes. Interestingly, treatment of adipocytes with RFE moved samples over along the PC1, strongly suggesting that RFE facilitates adipocyte differentiation.



Heatmap of the 500 topmost variable genes reveals a differentially-expressed gene signature between adipocytes and preadipocytes and indicates that RFE facilitates the progression of the pro-adipogenic program.

Pathway enrichment analysis further supports the role of RFE in inducing the adipogenic program.

KEGG		REAC		WP	
Term	P value	Term	P value	Term	P value
Metabolic pathways	2.467 e-06	Extracellular matrix organization	2.463 e-8	Cholesterol Biosynthesis	9.396 e-8
HIF-1 signaling pathway	4.617 e-05	Metabolism	3.585 e-06	Cholesterol metabolism (includes both Bloch and Kandutsch-Russell pathways)	2.347 e-7
Glycolysis / Gluconeogenesis	0.0015	Cholesterol biosynthesis	5.181 e-06	Focal Adhesion-PI3K-Akt-mTOR-signaling pathway	0.0330
Biosynthesis of unsaturated fatty acids	0.0027	Metabolism of carbohydrates	8.172 e-05	Glycolysis and Gluconeogenesis	0.0454
Steroid biosynthesis	0.0030	Collagen formation	0.00015		
Fatty acid metabolism	0.0039	Response to elevated platelet cytosolic Ca2+	0.0004		
PPAR signaling pathway	0.0099	Collagen biosynthesis and modifying enzymes	0.0004		
Glucagon signaling pathway	0.0154	Platelet degranulation	0.0009		
Axon guidance	0.0198	Crosslinking of collagen fibrils	0.0026		
PI3K-Akt signaling pathway	0.0329	Keratan sulfate degradation	0.0085		

Moreover, RFE induced the enrichment of several terms claiming remodeling of the extracellular space.