

Tamara Y. Forbes-Hernández, PhD student; Francesca Giampieri, PhD; Massimiliano Gasparrini, PhD student; Sadia Afrin, PhD student; Maurizio Battino, PhD, DsC, MD (Hon)

¹ Dipartimento di Scienze Cliniche Specialistiche ed Odontostomatologiche, Università Politecnica Marche, Ancona, Italy.

INTRODUCTION

Cardiovascular disease (CVD) is one of the major causes of mortality worldwide and its incidence has been increasing at an alarming rate. Dyslipidemia, including high concentrations of LDL- cholesterol and triglycerides, is a major risk factor for CVD. According to previous experience of our group, strawberries supplementation improves plasma lipids profile and biomarkers of antioxidant status in healthy volunteers, improving thus, some atherosclerotic risk factors. However the molecular mechanisms through which strawberry improves lipid profile remains unknown. In the present work we investigated whether strawberry would regulate hepatic lipid metabolism through AMP-activated protein kinase (AMPK) activation in HepG2 cells.

MATERIALS AND METHODS

Human HepG2 cells were cultured in DMEM, supplemented with 10% (v/v) FBS and penicillin-streptomycin (100 units/mL) at 37 °C and 5% CO₂. After reaching 80–90 % confluence, the cells were treated with various concentrations of strawberry dried methanolic extract or indicated reagents (compound C, lovastatin) for the indicated periods. After treatment, cells were lysed in lysis buffer and stored at -80 °C for further investigation. Cell viability was determined by the MTT assay and the cellular LDL- cholesterol and triglycerides content were measured using enzymatic colorimetric assay kits while total lipid accumulation was determined by the Oil Red O staining method. The effects of strawberry extract on AMPK pathway were determined by western blot analysis.

RESULTS

Dried methanolic extract (ug/mL)	Cell viability (%)		
	24h	48h	72h
0	100 ± 0.00	100 ± 0.00	100 ± 0.00
25	93 ± 3.96	86 ± 4.17	86 ± 3.74
50	91 ± 4.79	83 ± 2.46	84 ± 4.63
100	90 ± 3.76	81 ± 0.88	79 ± 7.88
250	87 ± 6.04	77 ± 5.45	73 ± 7.99
500	84 ± 3.76	72 ± 3.37	67 ± 5.48
1000	75 ± 2.06	65 ± 2.36	50 ± 2.17
2500	57 ± 0.87	55 ± 0.67	50 ± 2.12
5000	44 ± 3.52	44 ± 1.03	42 ± 5.37
7500	39 ± 2.35	43 ± 0.50	40 ± 5.44
10000	38 ± 1.31	40 ± 0.90	16 ± 1.66

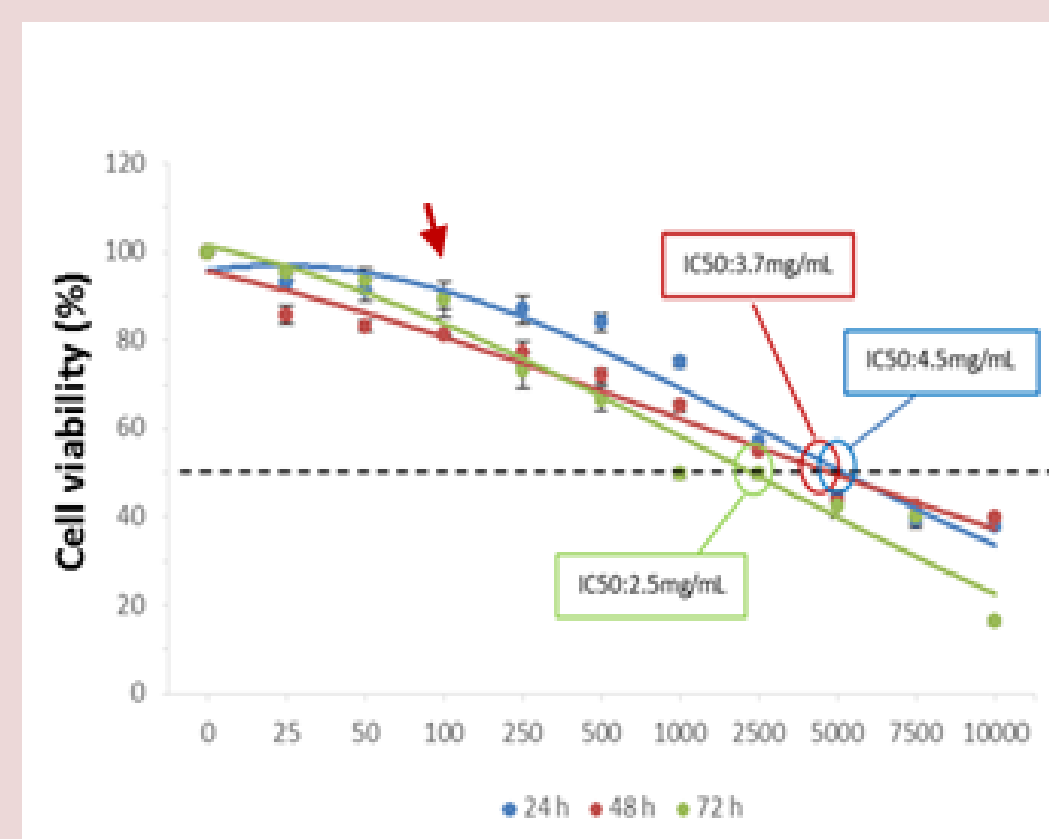


Figure 1. Viability of HepG2 cells after treatment with strawberry dried methanolic extract. Cells were treated at different concentrations of the extract for 24 , 48 and 72h. Values are expressed as mean ± SE (n = 3) of three independent experiments.

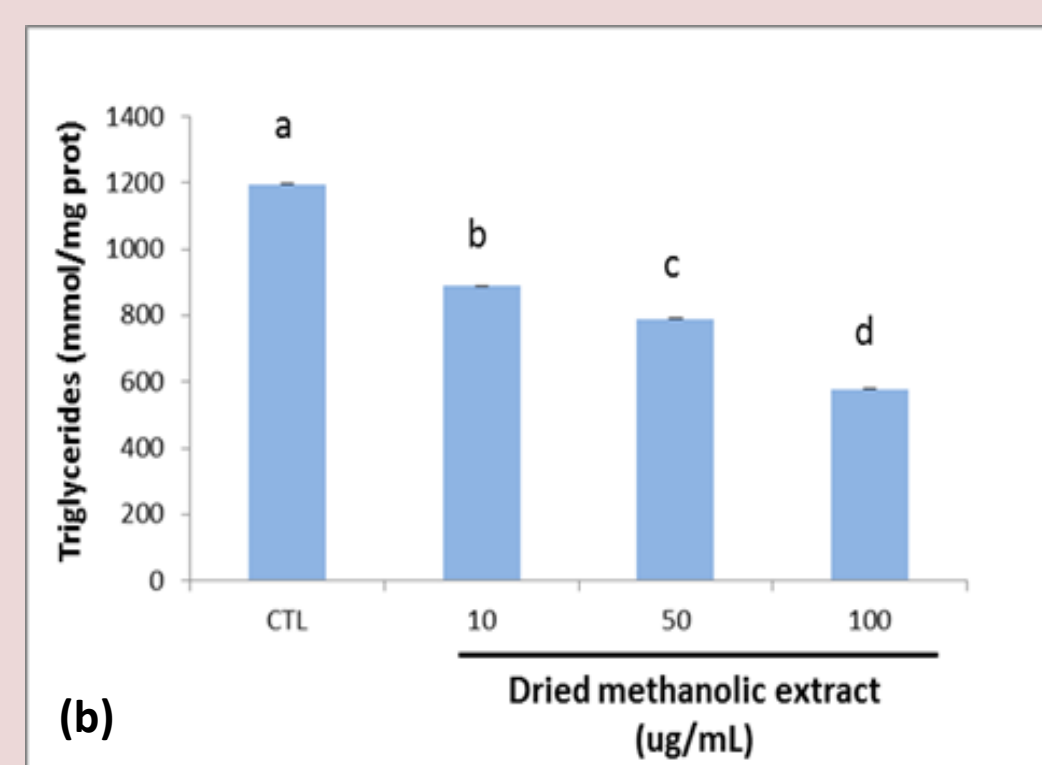
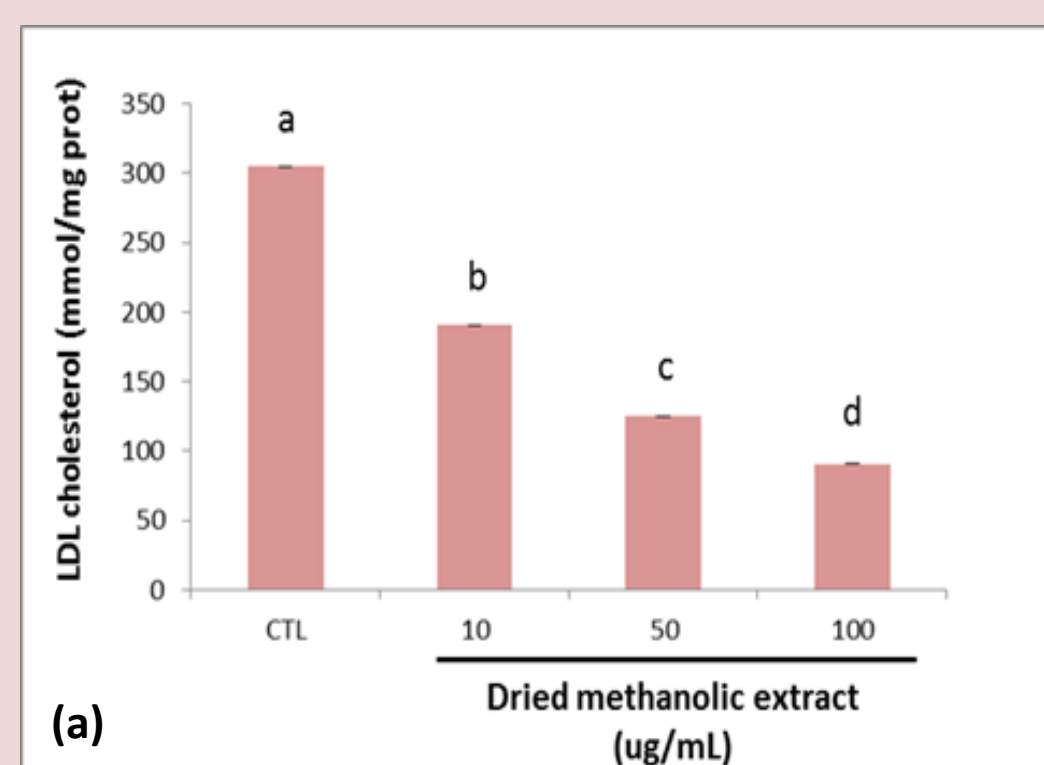


Figure 2. Effects of strawberry dried methanolic extract on (a) LDL-cholesterol and (b) triglycerides content. Cells were treated at different concentrations of the extract for 24 h. Values are expressed as mean ± SE (n = 3) of three independent experiments. Columns belonging to the same set of data with different superscript letters are significantly different (p < 0.05).

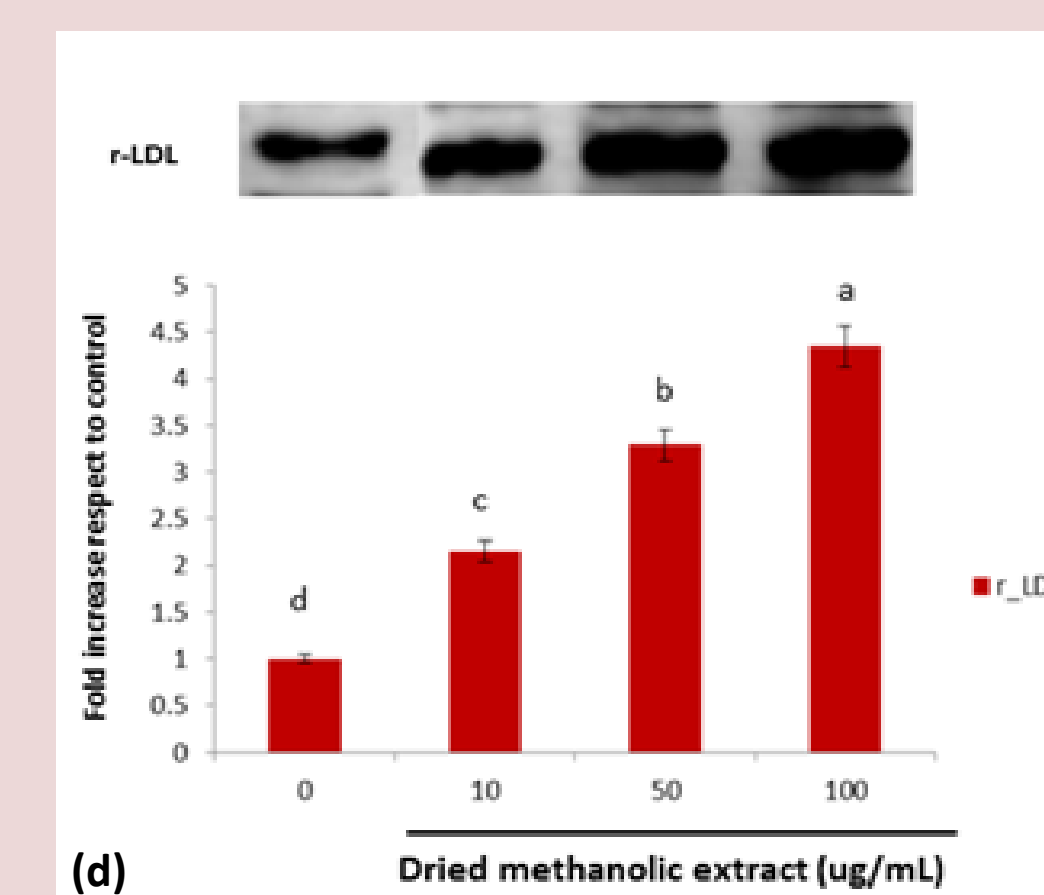
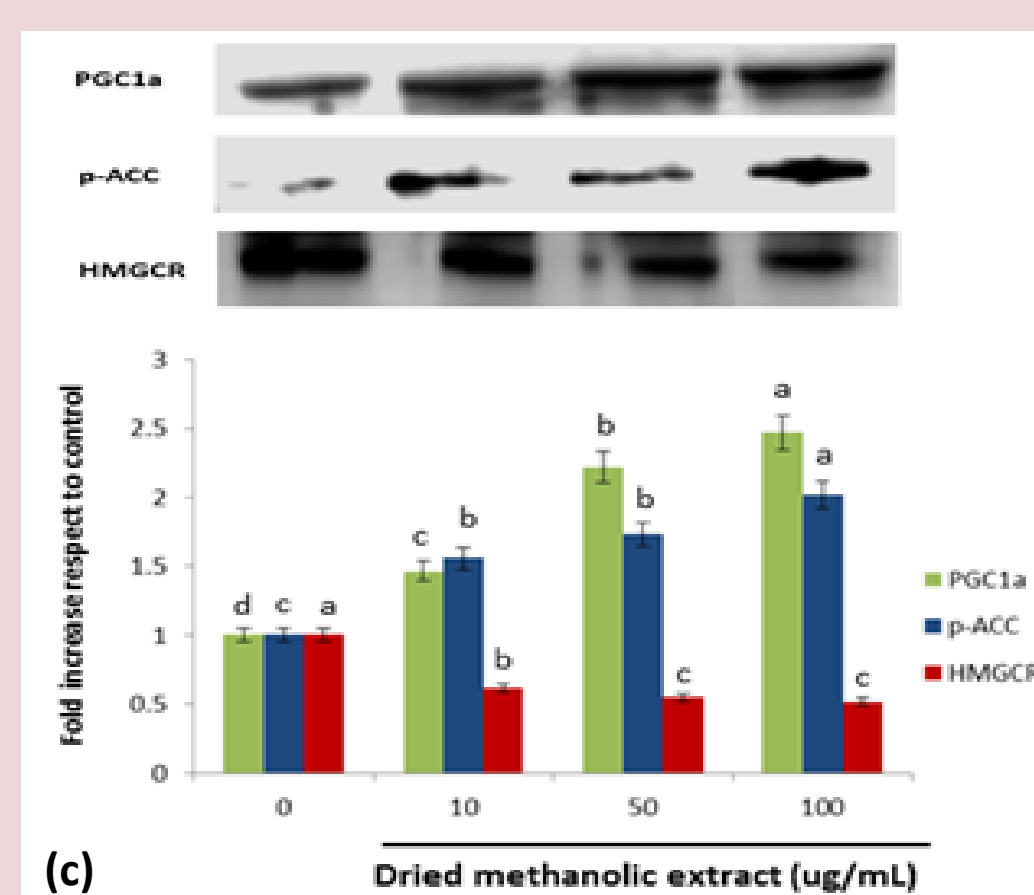
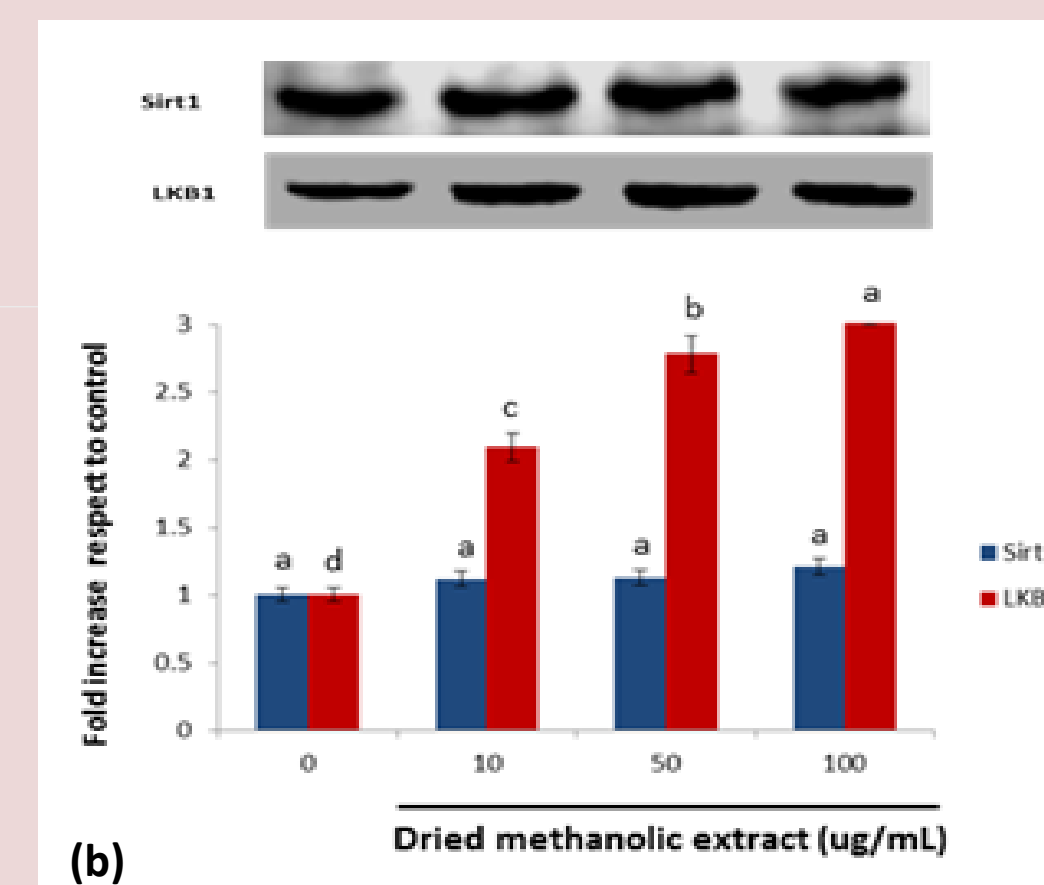
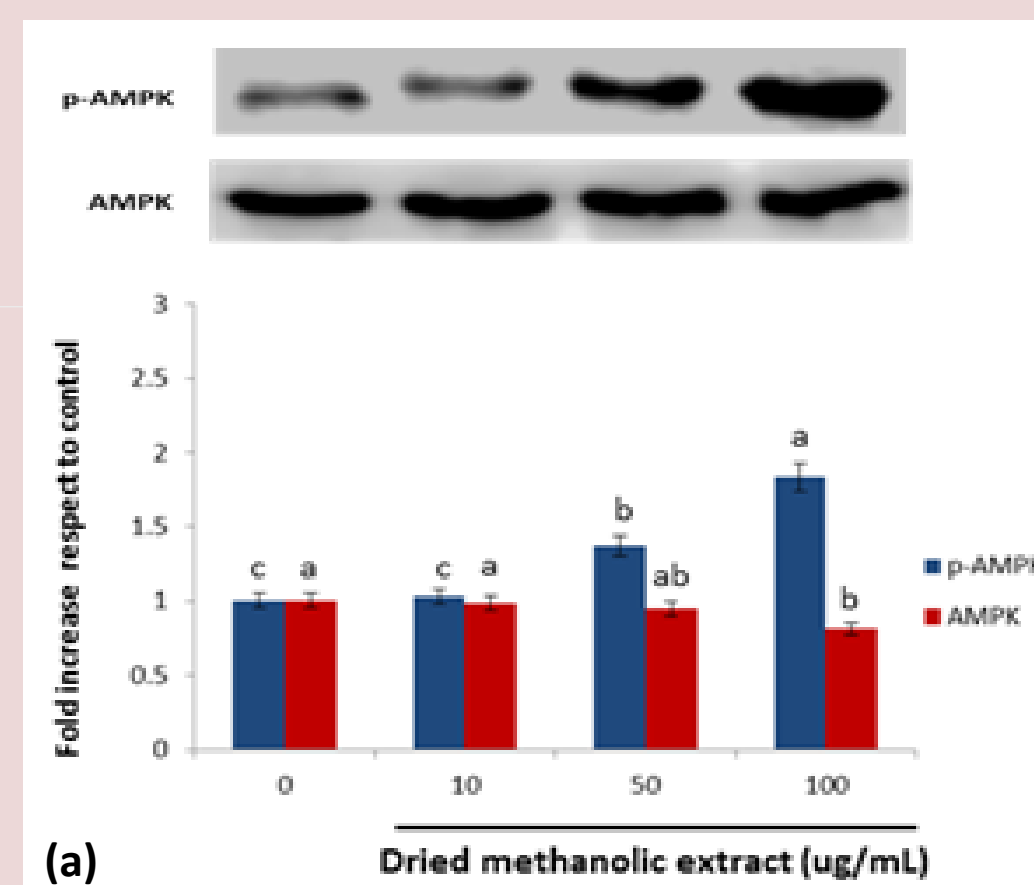


Figure 3. Effects of strawberry dried methanolic extract in the expression of AMPK pathway related proteins: (a) AMPK and p-AMPK (b) Sirt1 and LKB1 (c) PGC1α, p-ACC and HMGCR (d) r-LDL. Cells were treated at different concentrations of the extract for 24 h. Values are expressed as mean ± SE (n = 3) of three independent experiments. Columns belonging to the same set of data with different superscript letters are significantly different (p < 0.05).

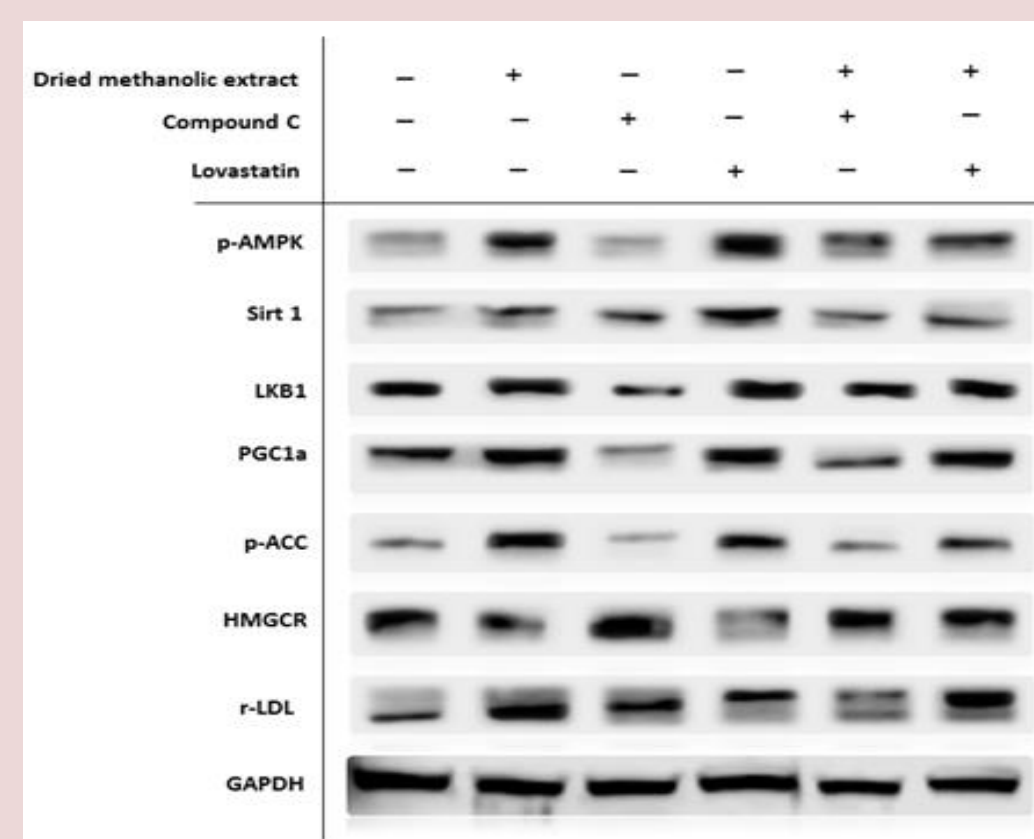


Figure 4. Effects of strawberry dried methanolic extract in the expression of AMPK pathway related proteins in the presence of Compound C (negative control) or Lovastatin (positive control). Cells were treated at different concentrations of the extract for 24 h.

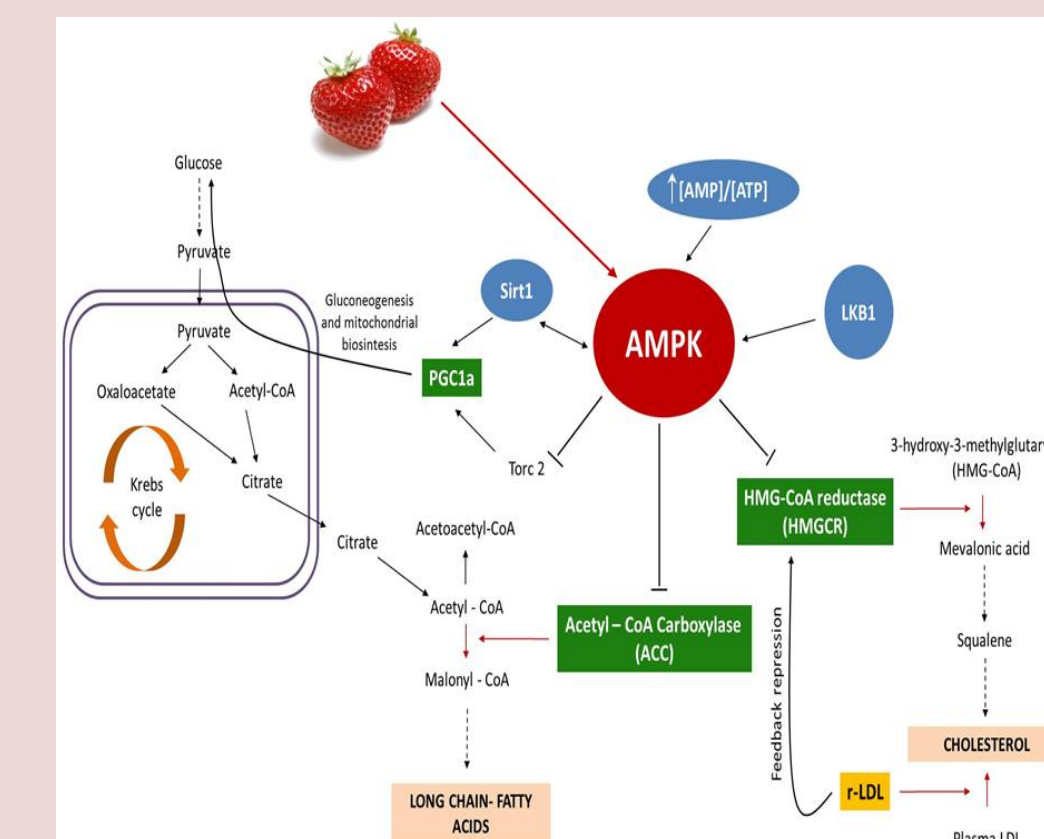


Figure 5. Proposed mechanism through which strawberry dried methanolic extract exerts its effects on lipid metabolism.

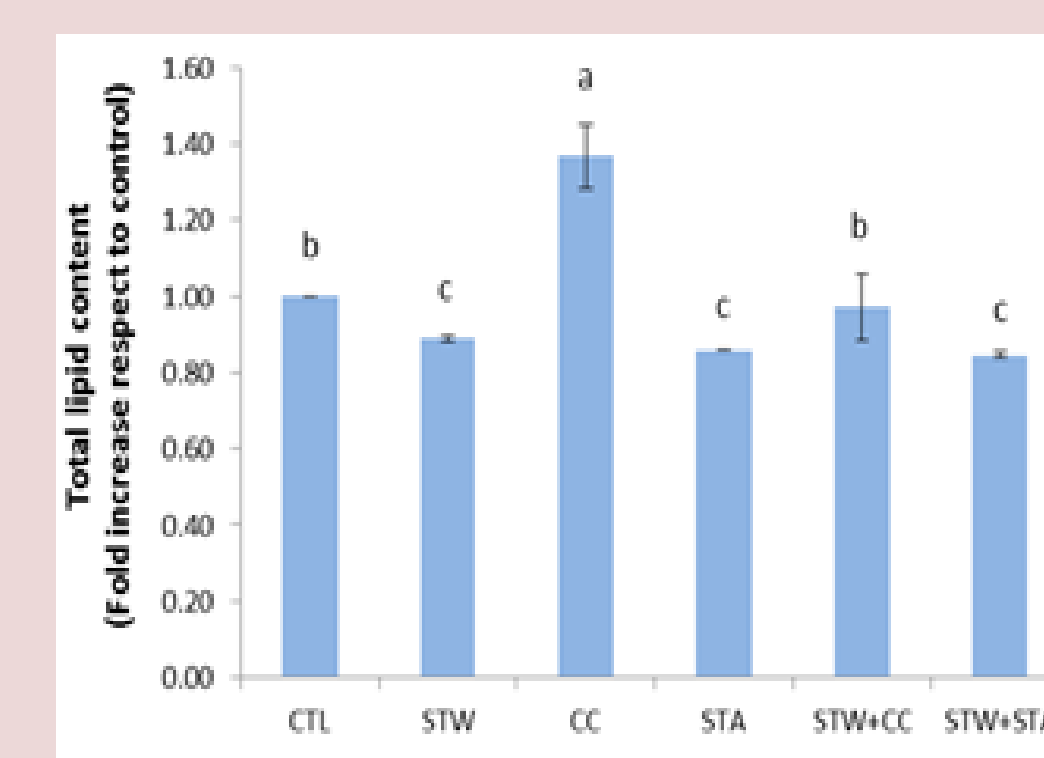
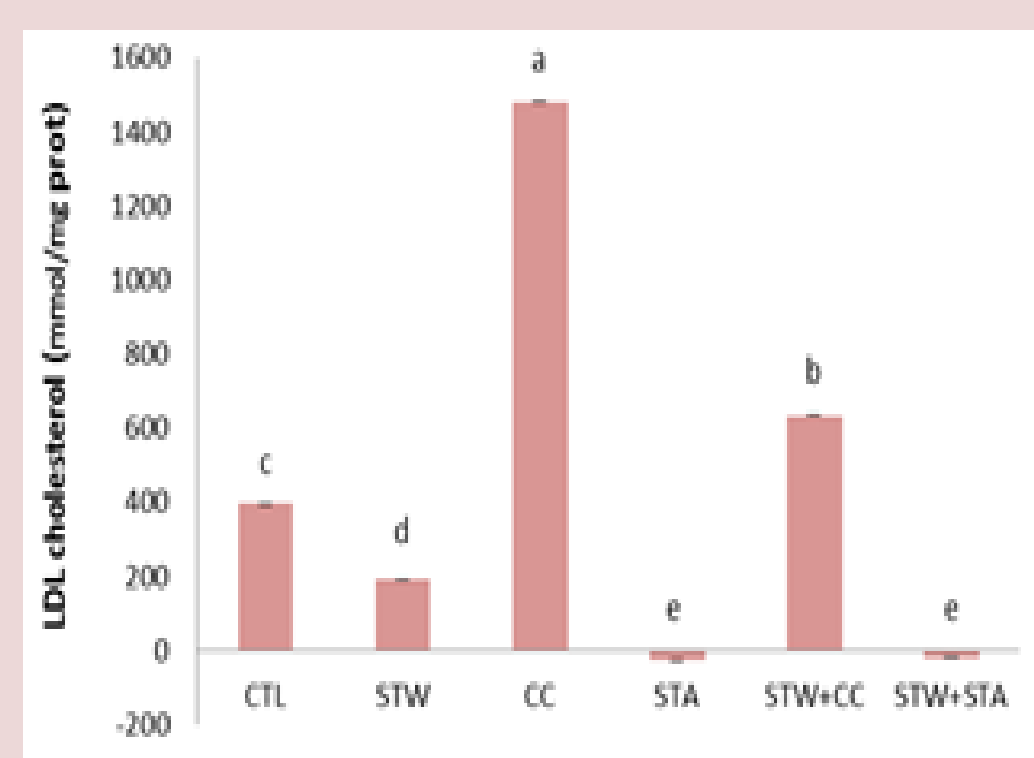


Figure 6. Effects of strawberry dried methanolic extract on (a) LDL-cholesterol levels and (b) total lipid content in the presence of Compound C (negative control) or Lovastatin (positive control). Cells were treated at different concentrations of the extract for 24 h.

CONCLUSIONS

Strawberry dried methanolic extract improved lipid profile in HepG2 cells by decreasing LDL- cholesterol, triglyceride levels and lipid accumulation. These effects depend on the regulation of AMPK pathway and activation of r-LDL.



Di chi sara' il mondo di domani?

Di chi oggi canta in coro.

17 GIUGNO 2015

IL Di.S.C.O. SI RACCONTA

