The effects of strawberry bioactive compounds on lipid metabolism and adipogenesis

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OBESITY is defined as abnormal or excessive fat accumulation that may impair health.

The fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended.
Most of the world’s population live in a country where there are more people overweight and obese than underweight.
Rising overweight (including obesity) rates in adults aged 15 -74 years

OBESITY RATES ARE EXPECTED TO INCREASE FURTHER
OVERWEIGHT AND OBESITY TRENDS IN THE WORLD

Rising overweight (including obesity) rates in adults aged 15 -74 years

OBESITY RATES ARE EXPECTED TO INCREASE FURTHER
In 2016, more than 1.9 billion adults were overweight and 650 million were obese.

At least 2.8 million people each year die as a result of being overweight or obese.

The prevalence of obesity nearly tripled between 1975 and 2016.

Once associated with high-income countries, obesity is now also prevalent in low- and middle-income countries.

By 2025 it is estimated that 2.7 billion adults and 268 million school-age children will be overweight or obese.
FACTS ABOUT OVERWEIGHT AND OBESITY

Globally, 41 million preschool children were overweight in 2016

- Overweight children are likely to become obese adults.
- They are more likely than non-overweight children to develop diabetes and CVDs at a younger age, which in turn are associated with a higher chance of premature death and disability.

Childhood obesity is one of the most serious public health challenges of the 21st century.
Obesity is a pandemic!!!!
FACTS ABOUT OVERWEIGHT AND OBESITY

Global Disability-Adjusted Life-Years and Deaths Associated with a High Body-Mass Index (2015)

The Consequences Of Obesity

- Coronary heart disease
- Type 2 diabetes
- Cancer (endometrial, breast, colon)
- Hypertension
- Stroke
- Liver disease
- Sleep apnea and respiratory problems
- Osteoarthritis
- Gynecological problems (abnormal menses, infertility)

OVERWEIGHT AND OBESITY ARE LINKED TO MORE DEATHS WORLDWIDE THAN UNDERWEIGHT
Risk factors of obesity

- Prevalence with Age
- Due to Medications, Medical Problems
- Lack of Sleep, Sleep Deprivation
- Being Physically Inactive
- Impact of Socio-Economic factors
- Unhealthy Diet
- Impact of Psychological factors
- With Family Lifestyle and Habits
- Role of Genetics

https://mumsfertility.com/overweight-obesity-causes-symptoms/
Health and nutrition

- Modernization
- Urbanization
- Demographic changes
- Socio-economic changes/economic growth
- Globalization of the world food market
- Media, marketing

Increased intake of calorie rich, low nutritive value foods: high intake of fats, trans fats, sugar, salt.
Health and nutrition
FRUIT AND VEGETABLE PROMOTION INITIATIVE / A MEETING REPORT / 25-27/08/03

- Up to 2.7 million lives could be saved annually with sufficient fruit and vegetable consumption.
- Low fruit and vegetable intake is among the top 10 selected risk factors for global mortality.

2.5 THE “5 A DAY PROGRAM” IN THE UNITED STATES OF AMERICA

Dr Lorelei DiSogra, Director, 5 A Day For Better Health Program, National Cancer Institute, Dr Frances Toccone, Director of Development, Produce for Better Health Foundation

2.6 THE EUROPEAN 5 A DAY-TYPE PROGRAMMES

Morten Strunge Meyer, Danish Cancer Society

2.7 SETTING UP A FRUIT AND VEGETABLE PROMOTION INITIATIVE IN A DEVELOPING COUNTRY

Dr Carlos A Monteiro, Centre for Epidemiological Studies in Health and Nutrition, School of Public Health, University of Sao Paulo
Mediterranean diet: a tool for primary prevention
Mediterranean Diet Pyramid: a lifestyle for today
Guidelines for Adult population

- Weekly
  - Potatoes ≤ 3s
  - White meat ≤ 2s
  - Fish/Seafood ≤ 2s
  - Dairy ≤ 2s (preferably low fat)
  - Olives / Nuts / Seeds ≤ 1 - 2s
  - Fruits 1 - 2 | Vegetables ≥ 2s
    (Variety of colours / textures
    (Cooked / Raw))

- Daily
  - Herbs / Spices / Garlic / Onions (less added salt)
  - Variety of flavours
  - Olive Oil
  - Bread / Pasta / Rice / Couscous / Other cereals ≤ 1 - 2s
    (preferably whole grain)
  - Water and herbal infusions

- Regular physical activity
- Adequate rest
- Conviviality

Serving size based on frugality and local habits
Wine in moderation and respecting social beliefs

Red meat ≤ 2s
Processed meat ≤ 1s
Eggs 2 - 4s
Legumes ≥ 2s
Sweets ≤ 2s

2010 edition

ICAF
International Commission on the Anthropology of Food and Nutrition

Public Health Nutrition: 14(12A), 2274 – 2284
doi:10.1017/S1368950210002515

Mediterranean diet pyramid today. Science and cultural updates
In the last 25 years...

- Nutritional and phytochemical characterization
- **In vitro** studies of anti-inflammatory, antitumor, antioxidant, and anti-atherosclerotic effects in various cellular models.
- **In vivo** studies with the aim of evaluating the effects on mitochondrial function, oxidative stress as well as on inflammatory, metabolic and apoptotic processes in animals and humans models.
Our findings...

- Anti-carcinogenic properties
- Anti-inflammatory properties
- Lipid-lowering effects
- Antioxidant activity
- Hypertension decrease
  - Total antioxidant capacity
  - Localization in cell membranes and lipoproteins domains
- Attenuation of inflammatory gene expression
  - Modulates iNOS activity
- Adiposity decrease
  - CRP (C-reactive protein)
  - α-glucosidase, α-amylase, pancreatic lipase
- Improvement of glycaemic profile
  - GLUT2 and SGLT
  - GLP-1 response
- Improvement of endothelial function
  - LDL oxidation
  - Central clustered platelets, VCAM-1, NADH oxidase
- Enhancement of lipid profile
  - Total and LDL cholesterol
Strawberry Romina

<table>
<thead>
<tr>
<th>Bioactive compounds</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Polyphenols (mg GAeq/g FW)</td>
<td>2.64 ± 2.63</td>
</tr>
<tr>
<td>Total Flavonoids (mg CATeq/g FW)</td>
<td>1.02 ± 0.87</td>
</tr>
<tr>
<td>Vitamin C (mg /g FW)</td>
<td>0.39 ± 0.23</td>
</tr>
<tr>
<td>Cyanidin-3-O-glucoside chloride (mg /g FW)</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>Pelargonidin-3-O-glucoside chloride (mg /g FW)</td>
<td>0.70 ± 0.25</td>
</tr>
<tr>
<td>Pelargonidin-3-O-rutinoside chloride (mg /g FW)</td>
<td>0.04 ± 0.08</td>
</tr>
<tr>
<td>Tetrahydrofolic acid (mg /g FW)</td>
<td>830.30 ± 5.04</td>
</tr>
<tr>
<td>5-methyl tetrahydrofolic acid (ng /g FW)</td>
<td>5.20 ± 1.12</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Fraction</th>
<th>FRAP (μmol Txeq/g)</th>
<th>DPPH (μmol Txeq/g)</th>
<th>ABTS (μmol Txeq/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh fruit</td>
<td>22.70 ± 2.03</td>
<td>8.11 ± 0.25</td>
<td>10.71 ± 0.58</td>
</tr>
<tr>
<td>Dried methanolic extract</td>
<td>168.25 ± 3.95</td>
<td>30.29 ± 0.18</td>
<td>35.51 ± 0.06</td>
</tr>
</tbody>
</table>


Lipid-lowering properties of strawberry polyphenols

Strawberry treatment improves lipid profile and antioxidant status in HepG2 cells

- Triglycerides, Total and LDL-cholesterol
- Lipid peroxidation

Lipid-lowering properties of strawberry polyphenols

Strawberry treatment improves lipid profile and antioxidant status in HepG2 cells

Intracellular ROS production

Antioxidant enzymes activity

Forbes et al., 2017 Int J Mol Sci, 18, 249
Lipid-lowering properties of strawberry polyphenols
Lipid-lowering properties of strawberry polyphenols

Expression of proteins related to the AMPK pathway

Lipid-lowering properties of strawberry polyphenols

How to confirm the involvement of AMPK pathway in the strawberry mechanisms of action?

**COMPOUND C**
pharmacological compound with the important function of blocking the phosphorylation of AMPK and, as a consequence, to inactivate this protein.

**LOVASTATIN**
member of the statins family, used to lower cholesterol and prevent cardiovascular diseases as a consequence of the inhibition of HMG-CoA reductase.

**COMPOUND C ≠ STRAWBERRY = LOVASTATIN**
Strawberry treatment has similar effects to the treatment with lovastatin and contrary to the obtained with Compound C regarding to lipid metabolism.
Lipid-lowering properties of strawberry polyphenols

Lipid-lowering properties of strawberry polyphenols

HOW CAN OBESITY BE DEFINED?

Abnormal or excessive fat accumulation.

Genetic Susceptibility + Positive Caloric Balance (overeating and sedentary lifestyle)

Increased adipose tissue

- Hypertrophy
- Hyperplasia

Adipogenic stimuli

C/EBPs/PPARγ

Adipocyte differentiation

Insulin sensitivity
Lipid accumulation
Adipokine secretion

Natural compounds

ABOISGENESIS

OBESITY
Inhibition of Adipogenesis and Induction of Apoptosis and Lipolysis by Stem Bromelain in 3T3-L1 Adipocytes

Sandeep Dave, Naval Jit Kaur, Ravikanth Nanduri, H. Kittorlal Dkhar, Ashwani Kumar, Pawan Gupta

Ursolic Acid Inhibits Adipogenesis in 3T3-L1 Adipocytes through LKB1/AMPK Pathway

Yonghun He, Ying Li, TianShen Zhao, Yanwen Wang, and Changhao Sun

Research Article

Antiobesity Effects of Unripe Rubus coreanus Miquel and Its Constituents: An In Vitro and In Vivo Characterization of the Underlying Mechanism

Doo-Eui Oh, Yujin Kim, Eun-Jin Choi, Houn-Mi Lee, Myung-A Jung, Donghyuck Bae, Ara Jo, Young Ran Kim, and Sunoho Kim

Reduction of lipid accumulation in white adipose tissues by Cassia tora (Leguminosae) seed extract is associated with AMPK activation

Thing-Fong Tseng, Hung-Jen Lu, Shihong-Shih Lin, Chiu Ju Chang, and I-Min Liu

α-Mangostin Induces Apoptosis and Suppresses Differentiation of 3T3-L1 Cells via Inhibiting Fatty Acid Synthase

Xiaofang Quan, Yi Wang, Xiaofeng Ma, Yan Liang, Wei Li, Qingyun Ma, Hezhong Jiang, and Yuanmin Zhou

Antiadipogenic Effect of Dietary Apigenin through Activation of AMPK in 3T3-L1 Cells

Makoto Ono and Ko Fujimoto

Combined Effects of Genistein, Quercetin, and Resveratrol in Human and 3T3-L1 Adipocytes

Hea Jin Park, Jeong-Yeh Yang, Suresh Ambati, Mary Anne Della-Fera, Dorothy B. Hausman, Srejana Rayalam, and Clifton A. Bailey

Caffeic acid methyl and ethyl esters exert potential antioxidant effects on glucose and lipid metabolism in cultured marine insulin-sensitive cells through mechanisms implicating activation of AMPK

Hoda M. El-Broshi, Farah Thong, Alan Nacher, and Pierre S. Haddad
HOW CAN OBESITY BE DEFINED?

Abnormal or excessive fat accumulation.

Could strawberries bioactive compounds regulate adipogenesis?
PROGRESSION OF 3T3-L1 PREADIPOCYTE DIFFERENTIATION

Pre-adipocytes expansion medium

3T3-L1 pre-adipocytes
(100% confluence)

Growing
Day -2

Differentiation medium
Day 0
IBMX (0.5 mM) + insulin (1 µg/mL) + Dexamethasone (1 µM)

Maintenance medium
Day 2
Insulin (1 µg/mL)

Differentiated
Day 10
3T3-L1 adipocytes

strawberry extract (STWE)

DNA replication

very early

early

intermediate

late

c-myc

c-fos

c-jun

LPL

confluence

MIX

DEX

insulin

commitment

i = 1 d

fat droplet formation

adipocyte-specific gene expression

cell division

growth arrest G0
**Evaluation of cell functions:**
- Viability assay
- Apoptosis
- Intracellular ROS production
- Antioxidant enzymes activities
- Mitochondrial functionality

**Adipogenic gene and protein expressions**

**Lipid metabolism:**
- LDL-cholesterol and TAGs contents
- Lipid peroxidation
- Total lipid accumulation

**EXPERIMENTAL DESIGN**

*Pre-adipocytes*

*Mature adipocytes*
EFFECTS OF STRAWBERRY EXTRACT (STWE) ON PROLIFERATION AND VIABILITY OF 3T3-L1 CELLS

No significant (toxic) effect on cell proliferation was observed at concentrations of STWE ≤100 μg/mL

Forbes-Hernandez et al., Food Funct., 2020, 11, 297-304
EFFECTS OF STWE ON MATURE ADIPOCYTES APOPTOSIS

Forbes-Hernandez et al., Food Funct., 2020, 11, 297-304
EFFECTS OF STWE ON 3T3-L1 CELLS DIFFERENTIATION

Inhibition of adipogenesis

Forbes-Hernandez et al., Food Funct., 2020, 11, 297-304
EFFECTS OF STWE ON LIPID ACCUMULATION

Decrease of total lipid content and improvement of lipid profile

Forbes-Hernandez et al., Food Funct., 2020,11, 297-304
EFFECTS OF STWE ON THE ADIPOGENIC TRANSCRIPTION FACTORS

Decrease of mRNA expression of C/EBP-α and PPAR-γ

Forbes-Hernandez et al., Food Funct., 2020, 11, 297-304
EFFECTS OF STWE ON THE DIFFERENTIATION RELATED TRANSCRIPTION FACTORS

Forbes-Hernandez et al., Food Funct., 2020, 11, 297-304
EFFECTS OF STWE ON THE DIFFERENTIATION RELATED TRANSCRIPTION FACTORS

Forbes-Hernandez et al., Food Funct., 2020, 11, 297-304
How to confirm the involvement of AMPK pathway in the strawberry mechanisms of action?

**COMPOUND C**

*pharmacological compound with the important function of blocking the phosphorylation of AMPK and, as a consequence, to inactivate this protein.*

**AMPK**

**Pre-adipocytes**

**Adipocytes**

**COMPOUND C ≠ STRAWBERRY**
EFFECTS OF AMPK INHIBITION IN THE EXPRESSION OF LIPOGENESIS AND FATTY ACIDS OXIDATION RELATED PROTEINS

Forbes-Hernandez et al., Food Funct., 2020, 11, 297-304
EFFECTS OF AMPK INHIBITION ON THE EXPRESSION OF LIPOGENESIS AND LIPID PROFILE

Forbes-Hernandez et al., Food Funct., 2020, 11, 297-304
EFFECTS OF STWE ON CELLULAR METABOLISM

Mitochondrial Respiration

Forbes-Hernandez et al., Food Funct., 2020, 11, 297-304
EFFECTS OF STWE ON CELLULAR METABOLISM

Glycolytic function

Forbes-Hernandez et al., Food Funct., 2020, 11, 297-304
EFFECTS OF STWE ON INTRACELLULAR ROS PRODUCTION

Forbes-Hernandez et al., Food Funct., 2020, 11, 297-304
EFFECTS OF STWE ON LIPID PEROXIDATION

Forbes-Hernandez et al., Food Funct., 2020,11, 297-304
EFFECTS OF STWE ON ANTIOXIDANT ENZYMES ACTIVITY AND PROTEIN EXPRESSION

![Graphs showing effects of strawberry extract on antioxidant enzymes activity and protein expression.](image-url)
EFFECTS OF STWE ON LIPID PRO-INFLAMMATORY CYTOKINES

Forbes-Hernandez et al., Food Funct., 2020, 11, 297-304

https://doi.org/10.3389/fendo.2013.00052
One-month strawberry-rich anthocyanin supplementation ameliorates cardiovascular risk, oxidative stress markers and platelet activation in humans

23 healthy volunteers  Ingestion of 500 g of strawberries

Strawberries consumption improves plasma lipids profile, biomarkers of antioxidant status, antihemolytic defenses and platelet function in healthy subjects.
STWE significantly reduced 3T3-L1 pre-adipocytes differentiation and lipid accumulation via the AMPK signaling pathway providing new insights into the molecular mechanism by which this STWE suppresses adipogenesis.

STWE supplementation significantly decreased ROS production and lipid peroxidation while increased antioxidant enzymes activities and expression in both pre-adipocytes and matures adipocytes.

It would be interesting to investigate whether strawberry extracts are equally efficient at all stages of differentiation or otherwise in one or more of them. STWE could be a promising naturally occurring therapeutic agent for the prevention and treatment of obesity.
Thank you for your kind attention