The potential of the *Vaccinium angustfolium* plant and its byproducts in countering diabetes and obesity

Pierre S. Haddad, Ph.D.
Department of Pharmacology
Université de Montréal, Montréal, Canada

Institute of Nutraceuticals and Functional Foods
Université Laval

Montreal Diabetes Research Center
Understand to Prevent and Cure

CIHR Team in Aboriginal Anti-Diabetic Medicines
Équipe IRSC sur les médecines autochtones antidiabétiques

WBANA Wild Blueberry Symposium
Quebec City, October 22, 2010

Outline

- Introduction
- Vaccinium angustifolium
  - Plant parts
  - Fermented berry juice
  - In vivo studies
- Acknowledgements

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Metabolic Syndrome and Type 2 Diabetes

Metabolic syndrome / Pre-diabetes

⇓

↓ Sensitivity to Insulin

⇓

Postprandial hyperglycemia

⇓

Compensatory hyperinsulinemia

⇓

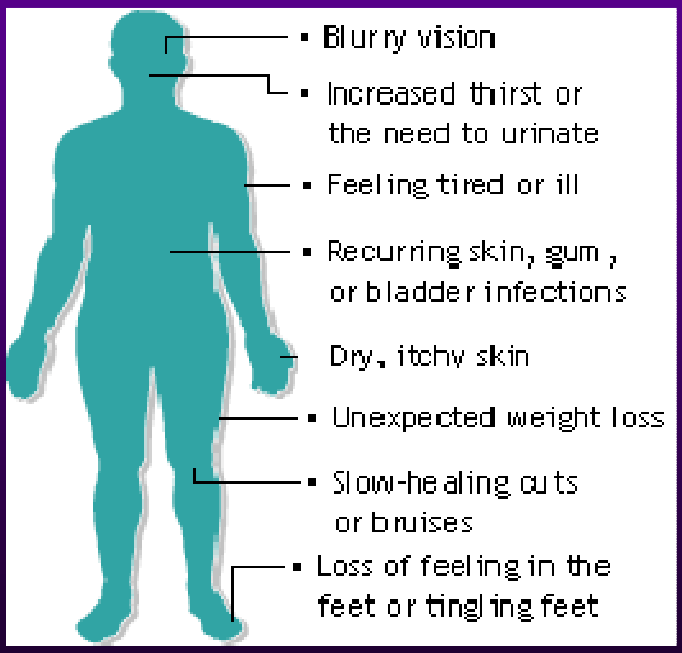
Pancreatic decompensation

⇓

Unregulated High Blood Sugar

⇓

Symptoms



Source: *Managing Type 2 Diabetes*. (2000). JAMA. Medem.

According to the WHO:

- Obesity/T2D = worldwide **epidemic**
- Problem amplified in **aboriginal populations**
- **Traditional medicine** and **NHPs** = first line of health care for 80% of the world's population **nowadays**
- 800-1200 antidiabetic NHPs reported worldwide (e.g. fenugreek, bitter gourd, prickly pear, ginseng, etc.)

But…

- Little **evidence-based research** (however 80% NHPs tested have promising biological activity)
- Problems = **product quality**: e.g. Botanical ID, source, preparation, dose

Our work:

- Mediterranean Nigella (*Nigella sativa*)
- Moroccan Argan oil (*Argania spinosa*)
- Canadian lowbush blueberry (*Vaccinium angustifolium*)
- Cashew seed (*Anacardium occidentale*)
- Boreal forest plants (Cree Traditional Medicine)

Recommanded NHPs for Type 2 Diabetes

Results from a survey in Quebec, Canada

Rank	Plant	Latin Name	Citations	TCPT	% 1st choice
1	Blueberry	*Vaccinium myrtillus/augustifolia*	15	12,00	33,3%
2	Fenugreek	*Trigonella foenum graecum*	9	9,00	25,0%
3	Dandelion	*Taraxacum officinale*	6	3,12	8,7%
4	Milk Thistle	*Silybum marianum*	3	2,50	6,9%
5	Gymnema	*Gymnema sylvester*	3	2,50	6,9%
6	Licorice	*Glycyrrhiza glabra*	3	1,75	4,9%
7	Artichoke	*Cynara scolymus*	3	1,70	4,7%
8	Jambolan	*Syzygium cumini*	2	1,50	4,2%
9	Prickly Pear	*Opuntia streptacantha*	2	1,50	4,2%
10	Ginseng	*Panax ginseng/Ginseng quinquefolium*	3	1,33	3,7%

Haddad et al. *Diabetes Care* 24:608-609, **2001**

Haddad et al. *J. Herbs Spices Med. Plants* 10:25-45, **2003**

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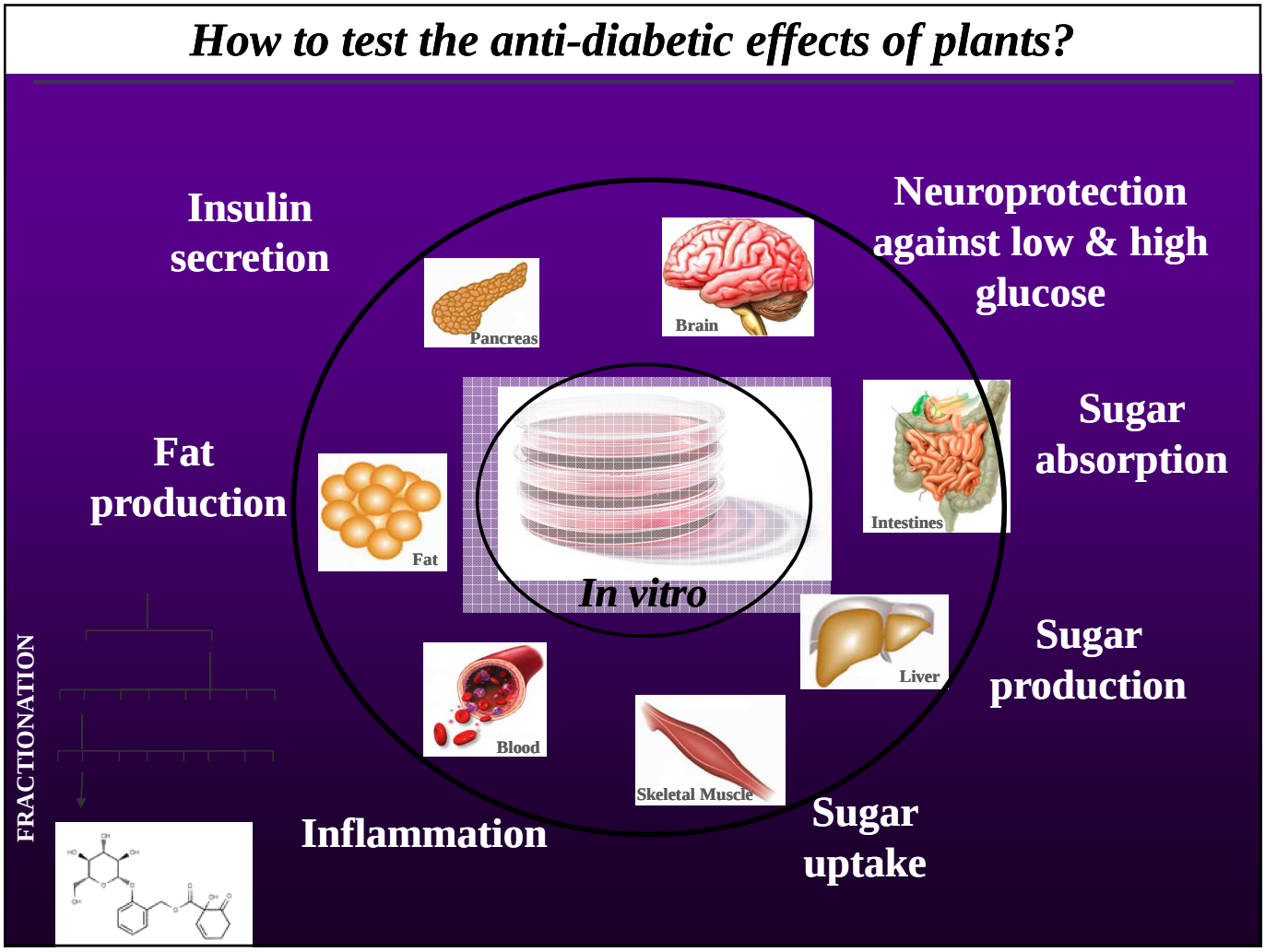
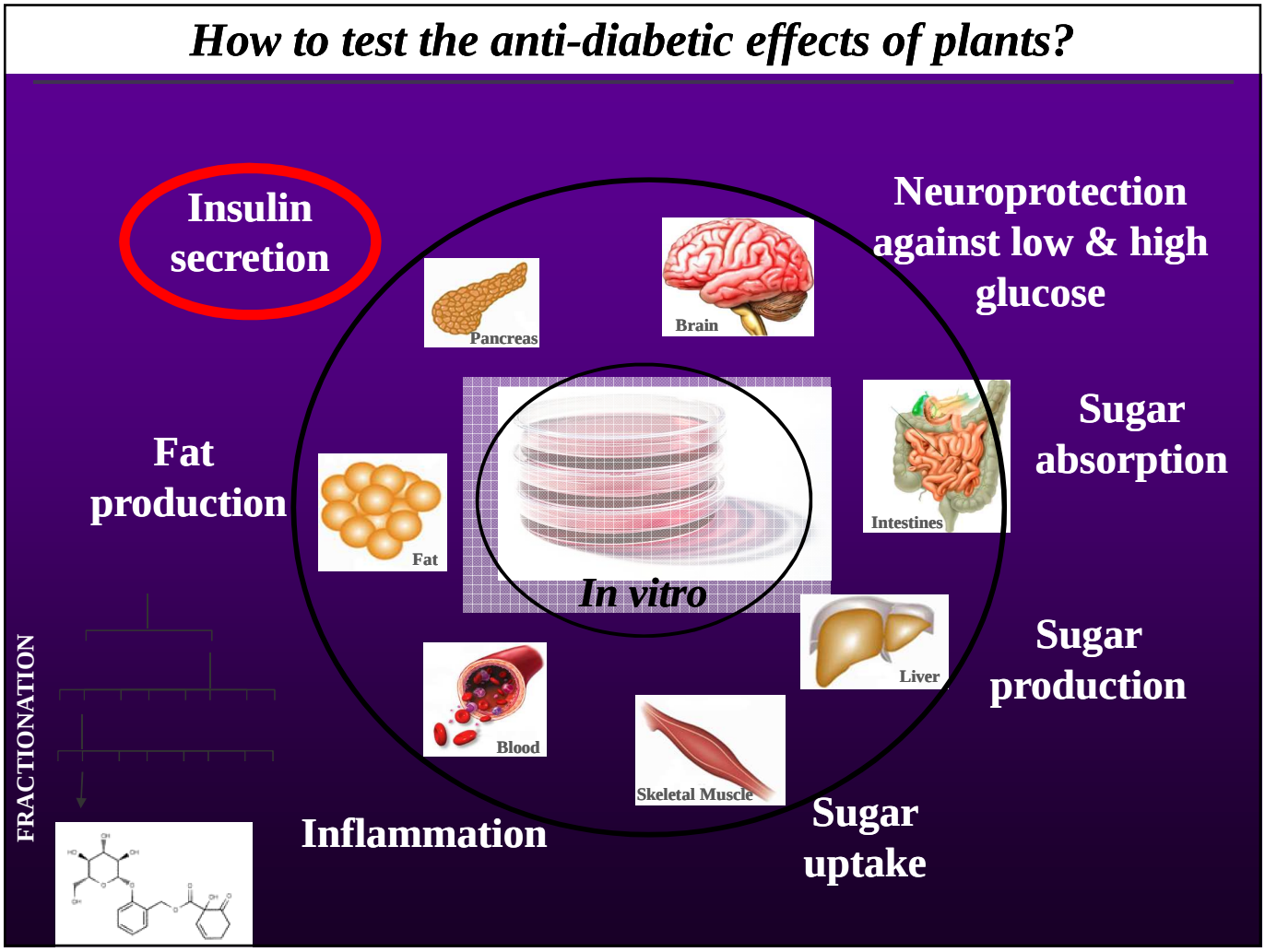
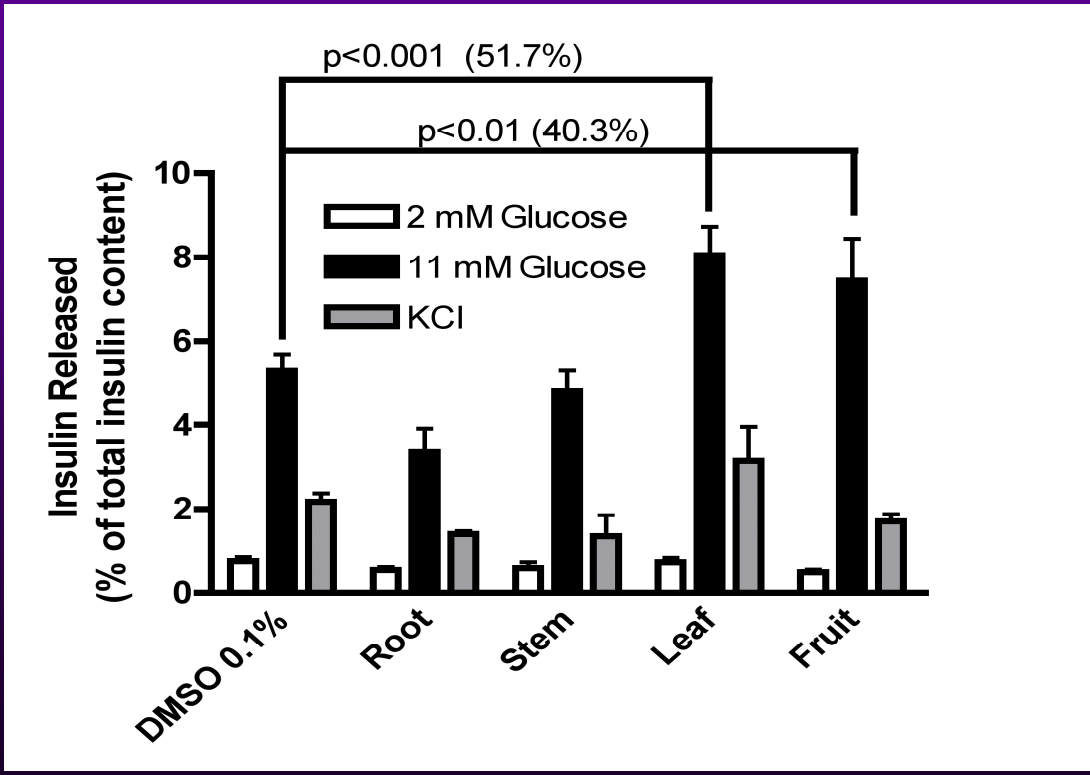
How to test the anti-diabetic effects of plants?



How to test the anti-diabetic effects of plants?

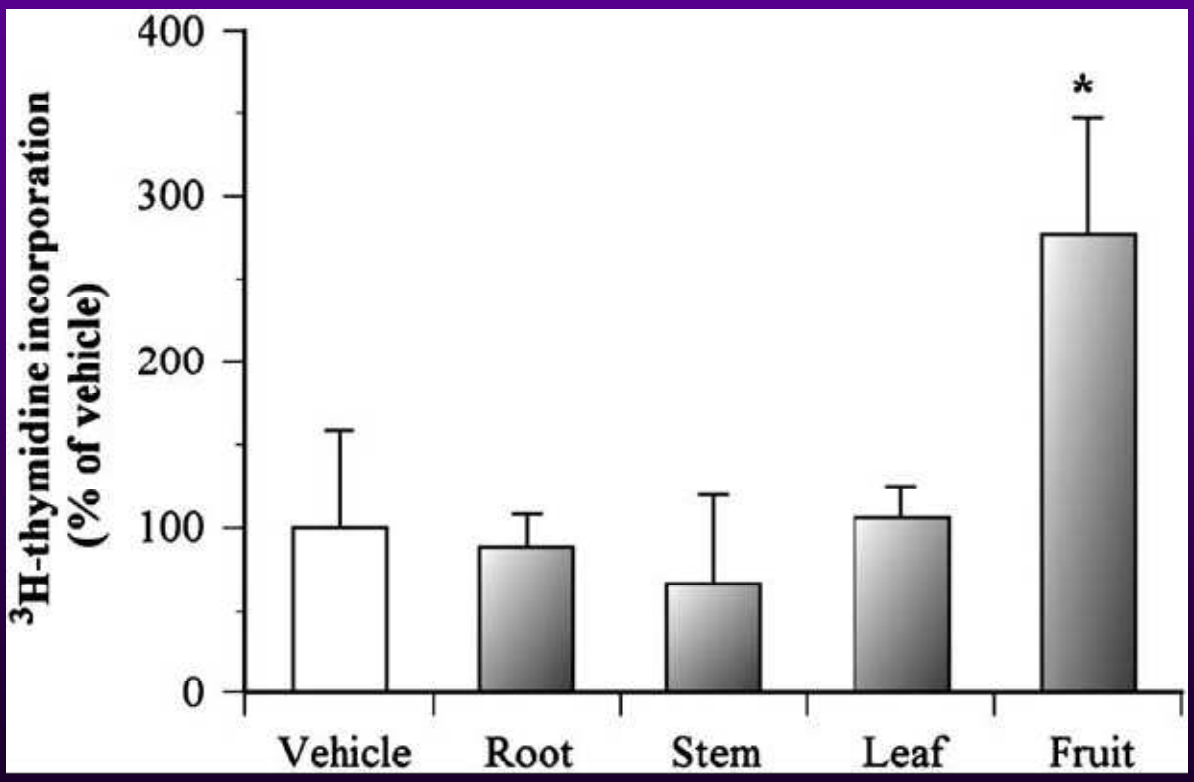


Effect on Insulin Secretion in INS 832/13 Beta Cells:



Martineau et al., Phytomedicine 13:612-623, 2006

Effect on Beta Cell Proliferation in β-Tet cells:



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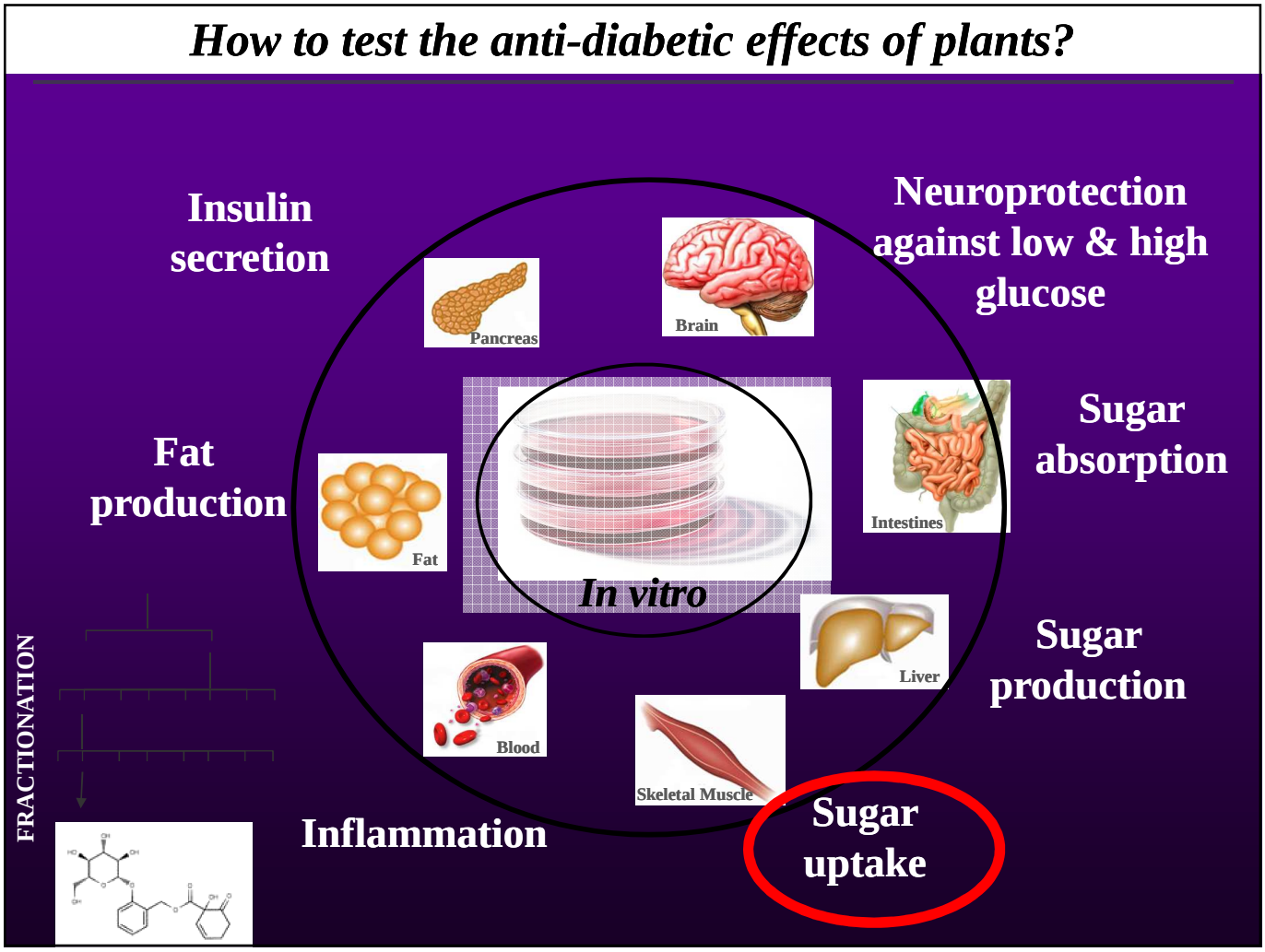
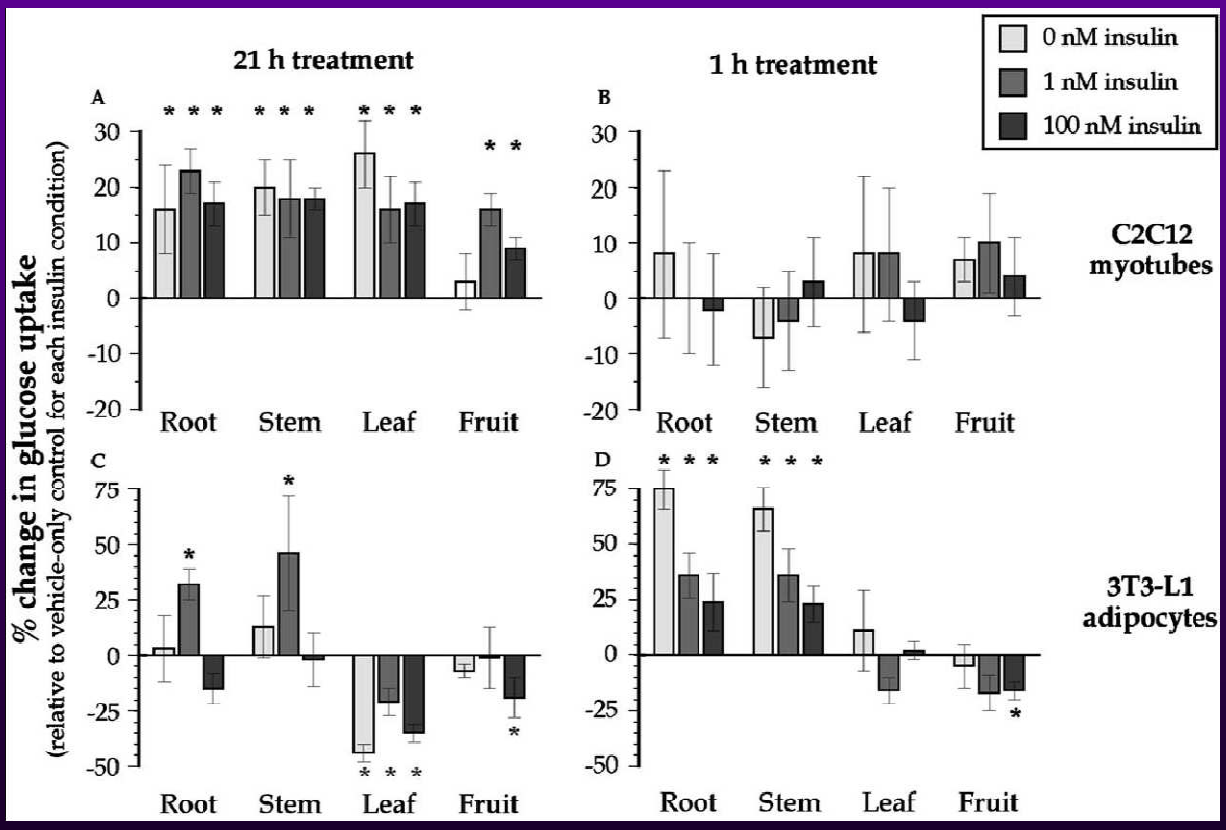


Effects on Glucose Uptake



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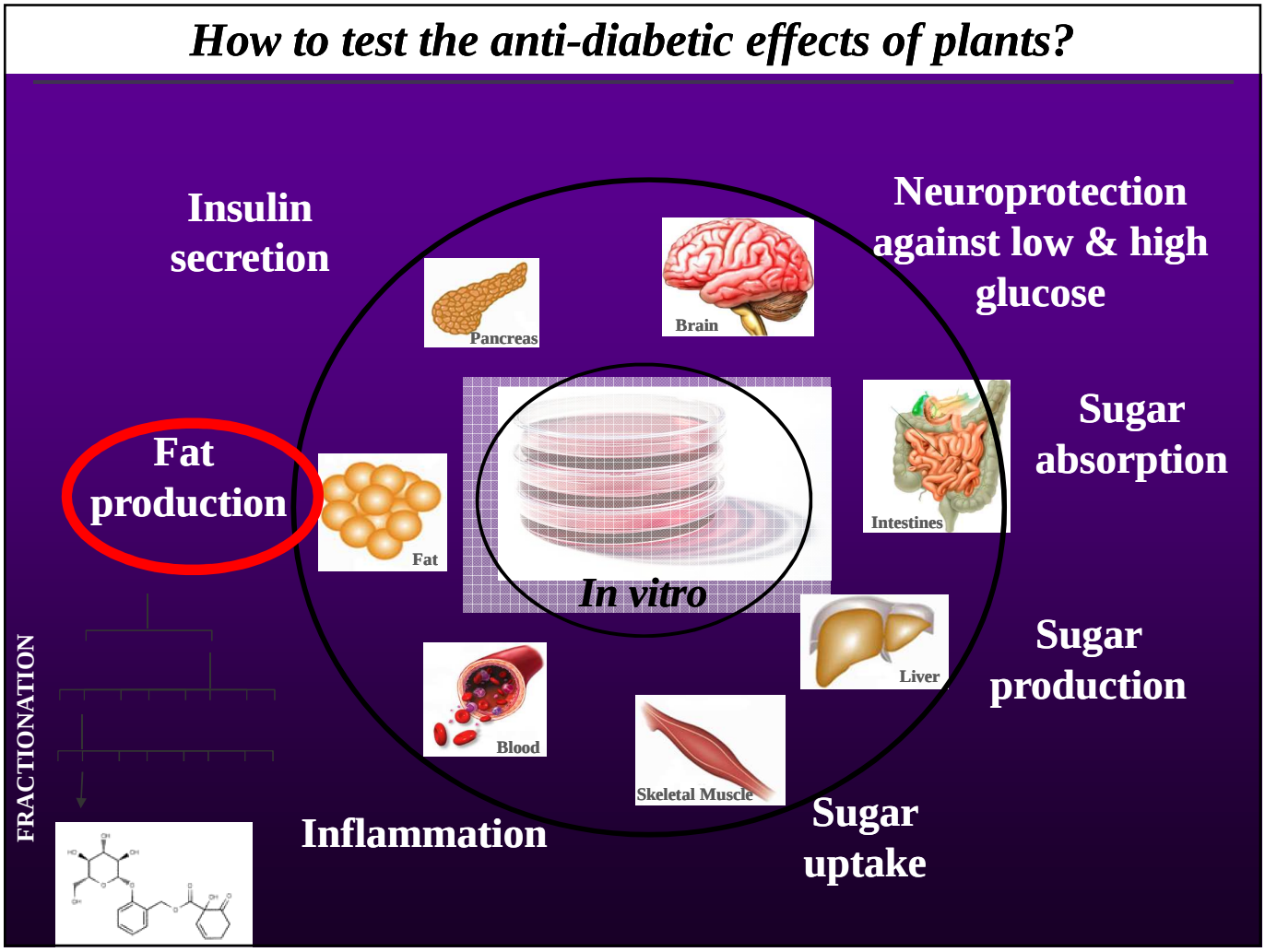
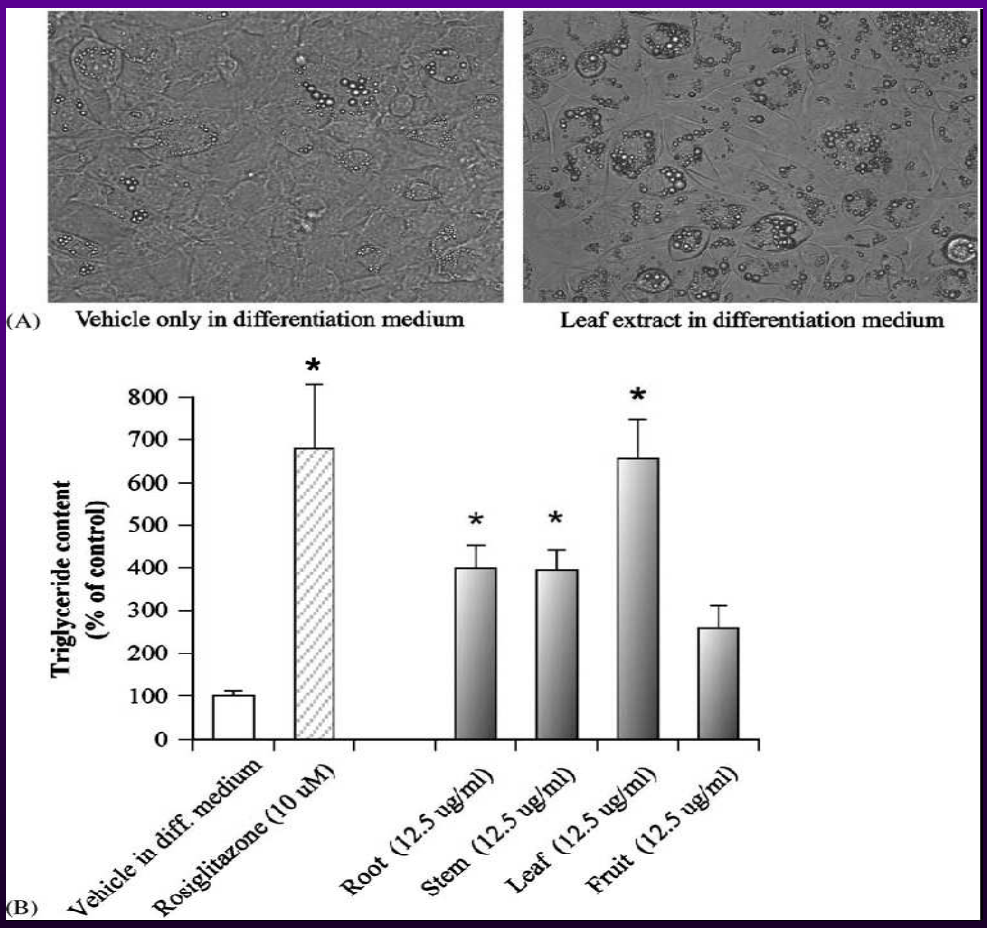


Effects on Differentiation in 3T3-L1 Adipocytes :



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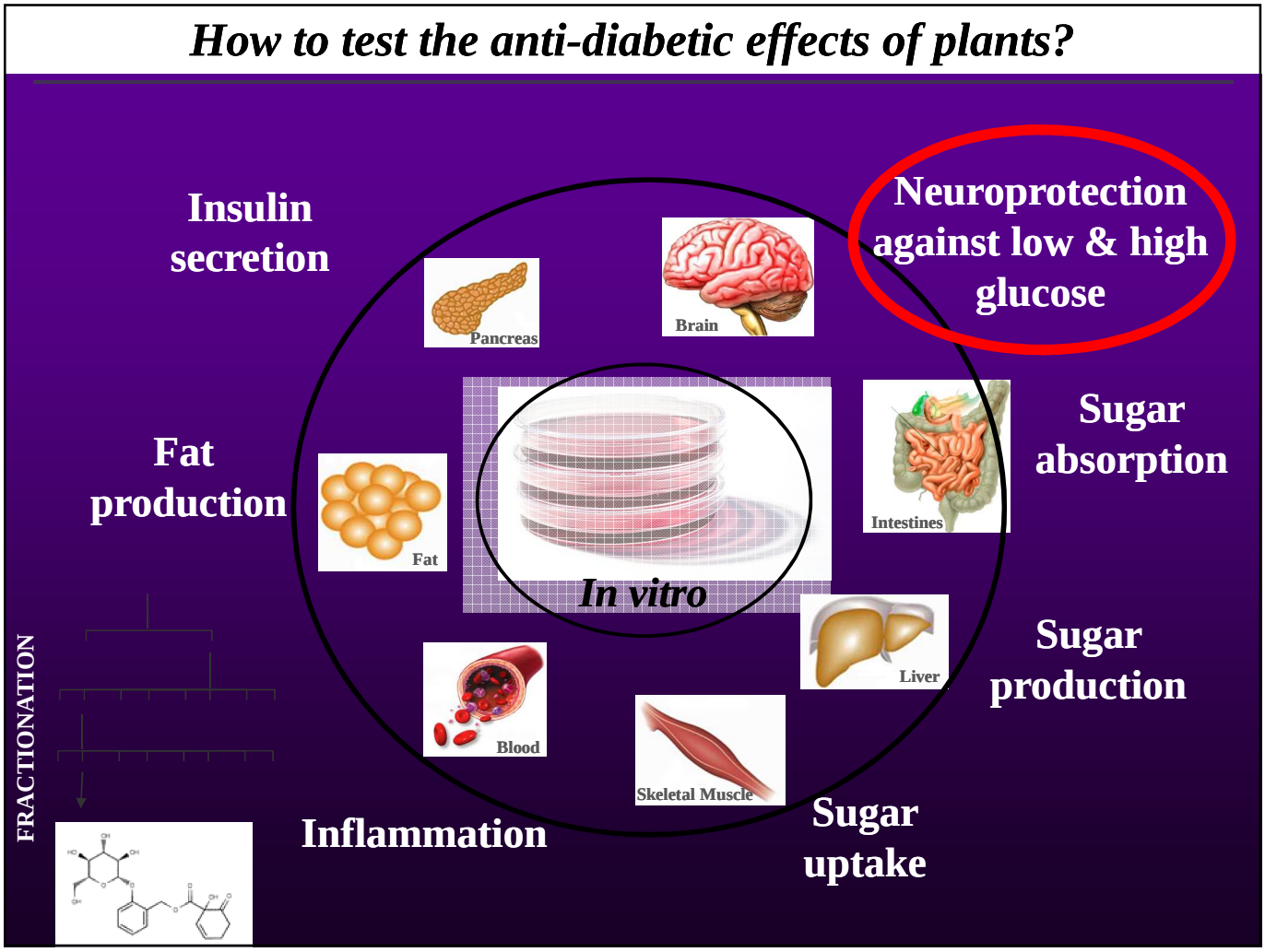
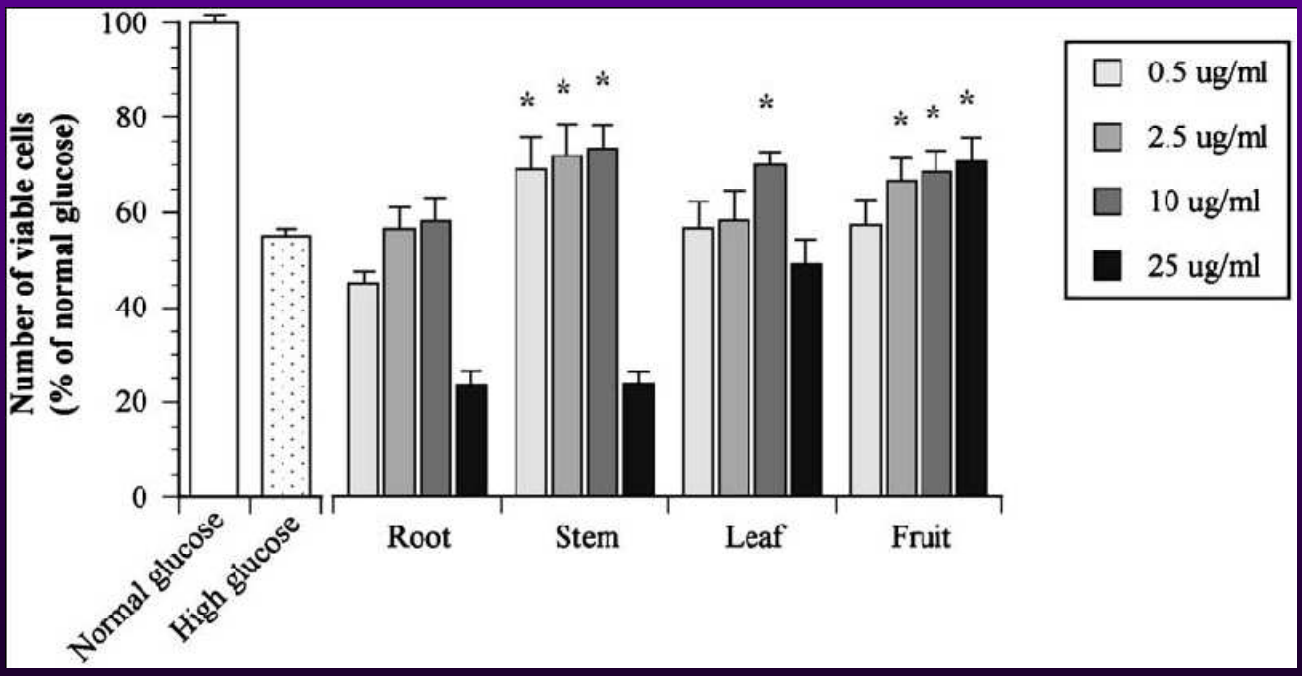


Cytoprotective Effect Against Hyperglycemia in PC12 Cells :



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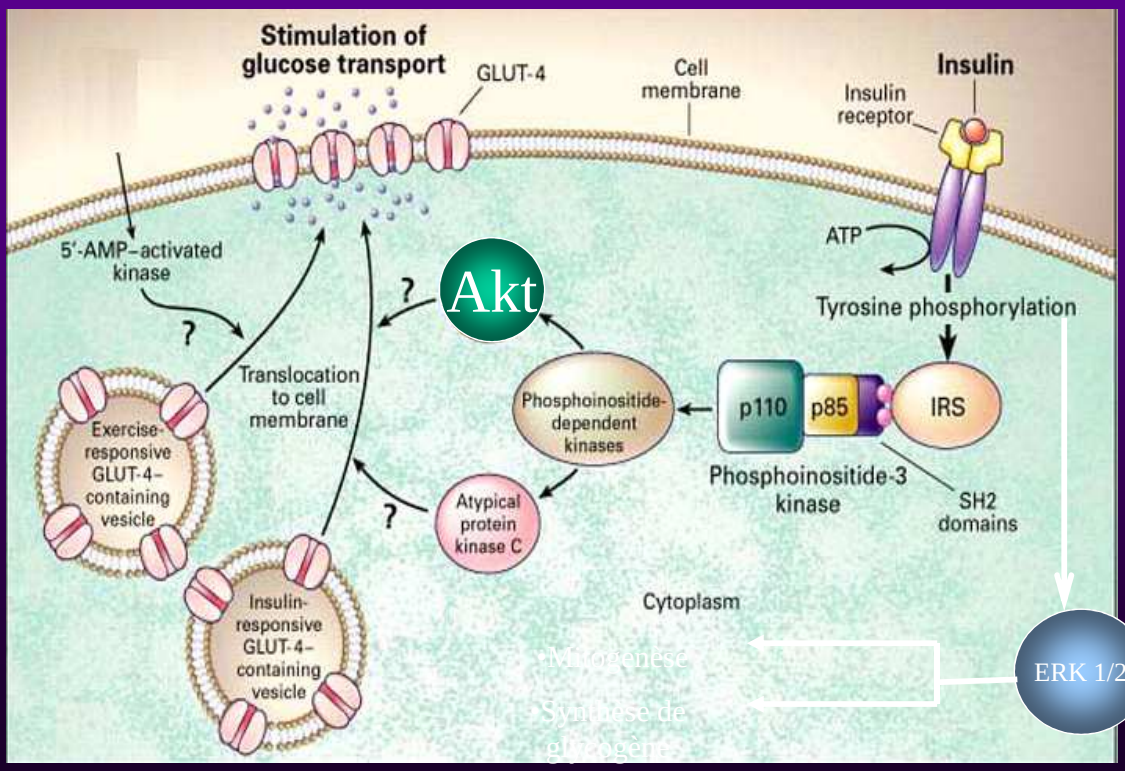
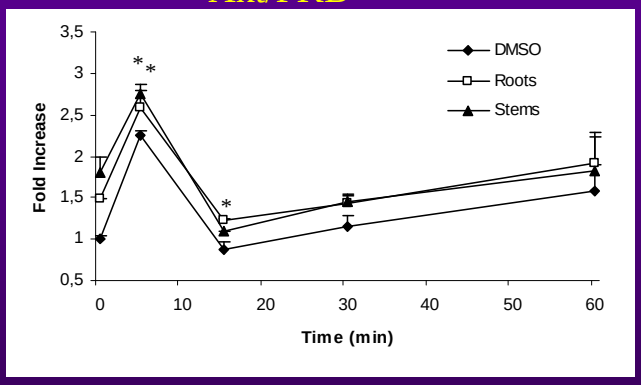
Insulin Signaling

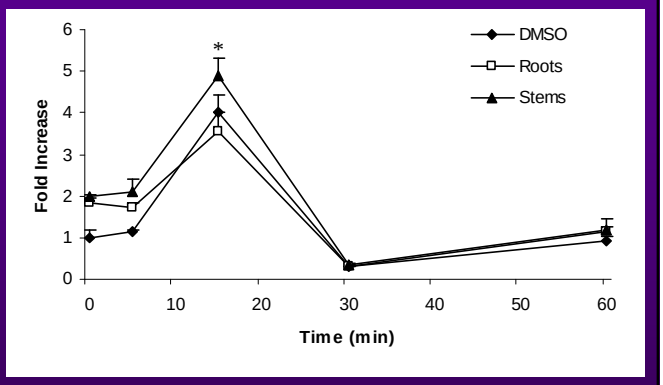


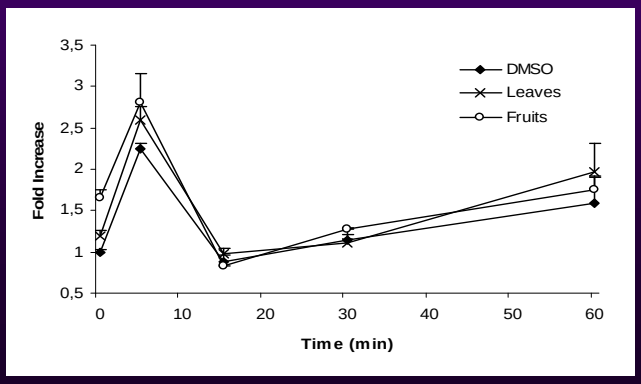
Effect on Insulin Signaling in 3T3-L1 Adipocytes:

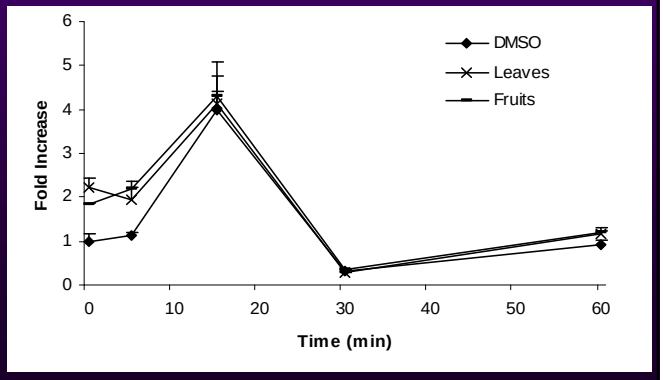
Akt/PKB



ERK 1/2 MAPK







SUMMARY

Ethanolic extracts of *V. angustifolium* show interesting cytoprotective and anti-diabetic potential:

- Insulino-mimetic and insulin-sensitizing activity on glucose transport in muscle and fat cells
  - Roots ≅ Stems > Leaves > Fruits
- Increase in basal and insulin-stimulated Akt/PKB and Erk1/2 MAPK (muscle, fat, liver)
  - Roots ≅ Stems > Leaves > Fruits
- Stimulation of pancreatic insulin secretion
  - Fruits ≅ Leaves >> Stems ≅ Roots
- Stimulation of fat cell differentiation (glithazone-like PPARγ agonist?)
  - Leaves >> Roots ≅ Stems > Fruits
- Neuroprotection: Stems > Fruits > Leaves > Roots

Conclusions

- Validation of the traditional medicinal use of *Vaccinium angustifolium* roots and leaves against diabetes
- Suggestion that stems may be as pharmacologically active as roots → important for sustainable use and commercial diversification of blueberry byproducts
- Combination of stem and leaf extracts may be beneficial → complementary mechanisms of action
- Canadian blueberry could be beneficial as a complementary treatment for type 2 diabetes.

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Transformation of blueberry juice

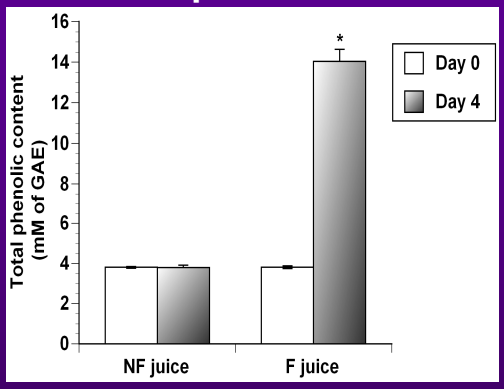
Serratia vaccinii

\+

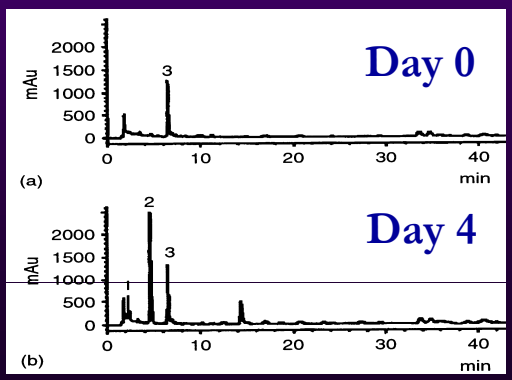
4 days fermentation

Lowbush blueberry juice (*Vaccinium angustifolium*. Ait)

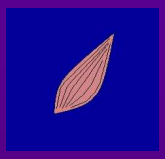
Increased phenolic content



Production of two new compounds

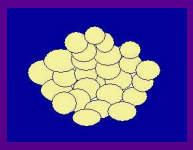


Increase of glucose uptake in muscle cells and adipocytes



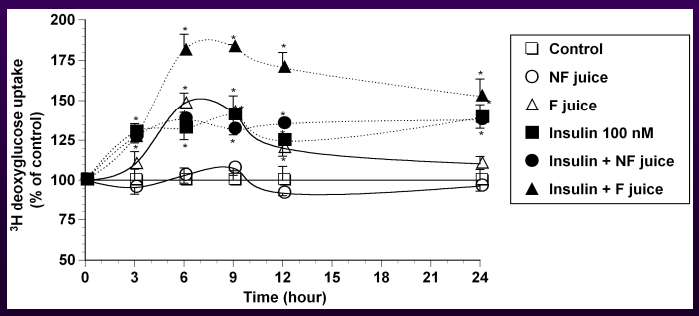
Skeletal Muscle (C2C12 myotubes)

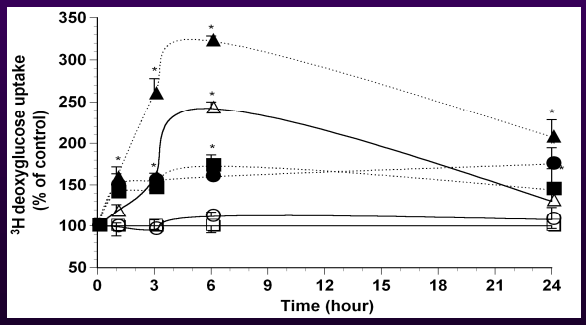
Adipose Tissue (3T3-L1 adipocytes)



Differentiated cells incubated 1 h to 24 h with blueberry juices at 30 µM GAE

Rates of basal and insulin-stimulated glucose uptake measured by ^{3}H-deoxyglucose transport assay

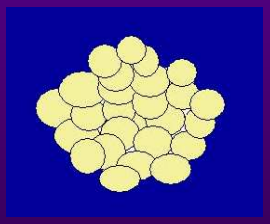




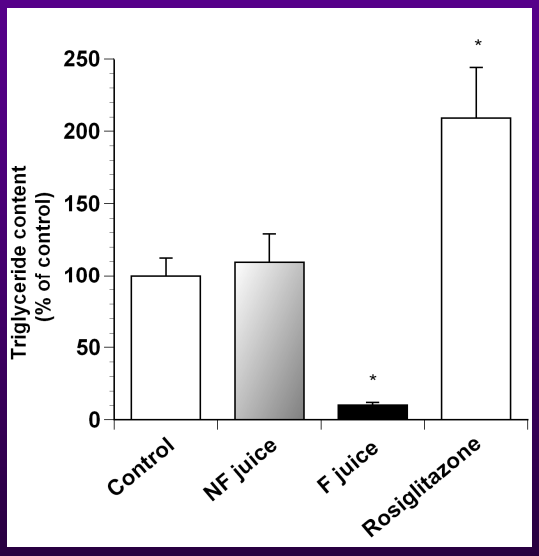
Vuong et al., Can. J. Physiol. Pharmacol. 85:956-965, 2007

Inhibition of adipogenesis and of PPAR-γ

3T3-L1 pre-adipocytes



Differentiation in the presence of blueberry juices at 30 µM GAE



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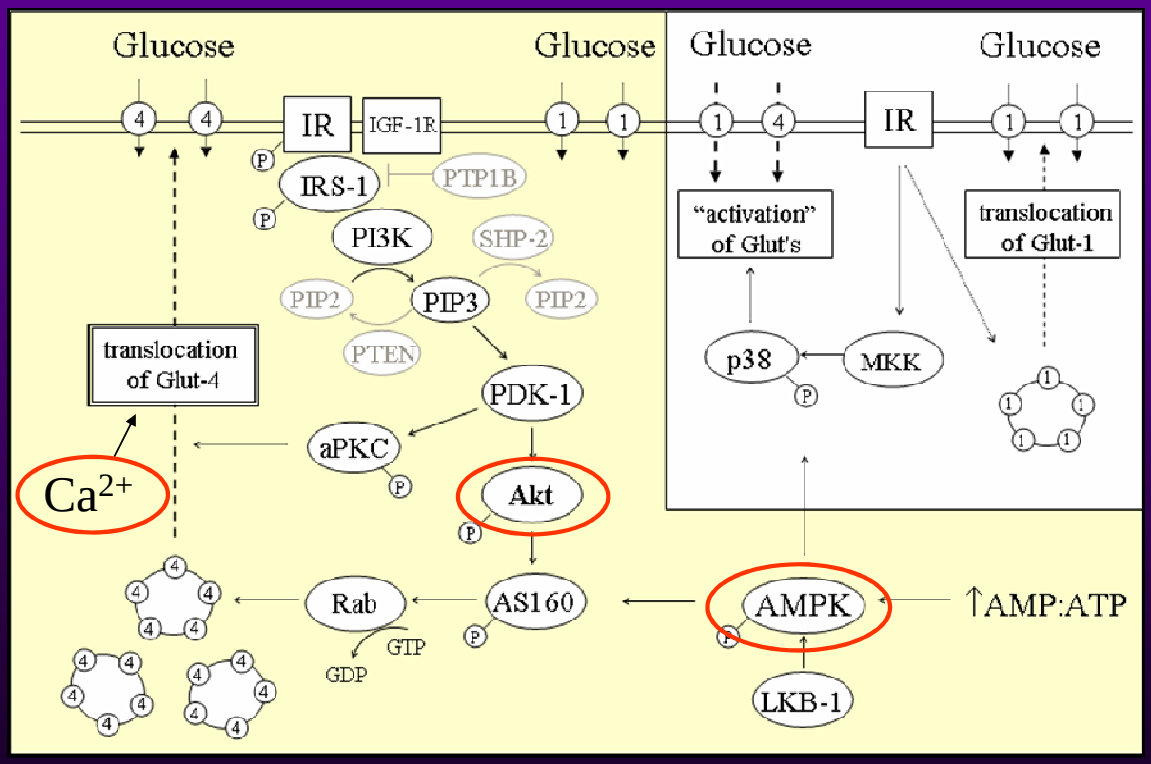
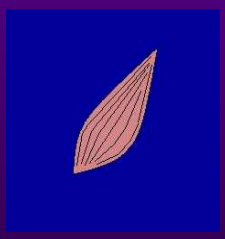
Insulin and non-insulin dependent signaling pathways:

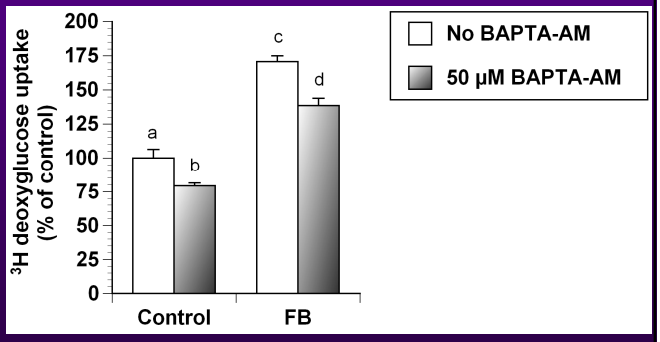


Lack of contribution of cytosolic Ca^{2+} on glucose uptake

Skeletal Muscle

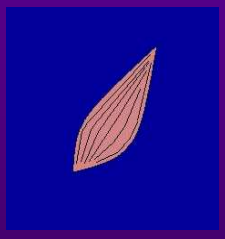


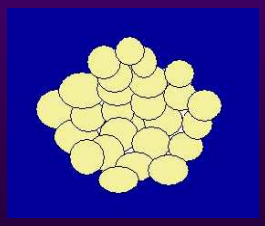
Ca^{2+}-chelator BAPTA-AM
+ blueberry juice



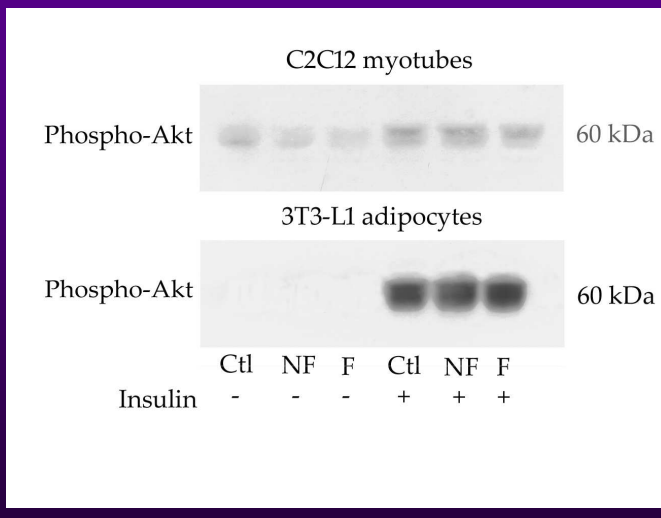
Vuong et al., Can. J. Physiol. Pharmacol. 85:956-965, 2007

Lack of blueberry juices effect on insulin-dependant pathway





6 h treatment with blueberry juice in the presence/absence of insulin



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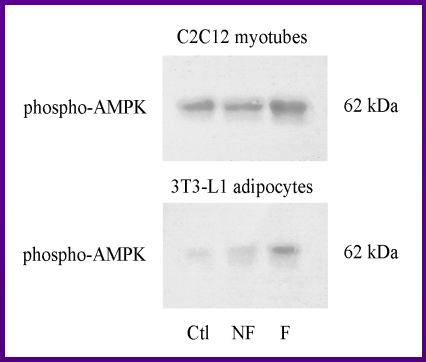
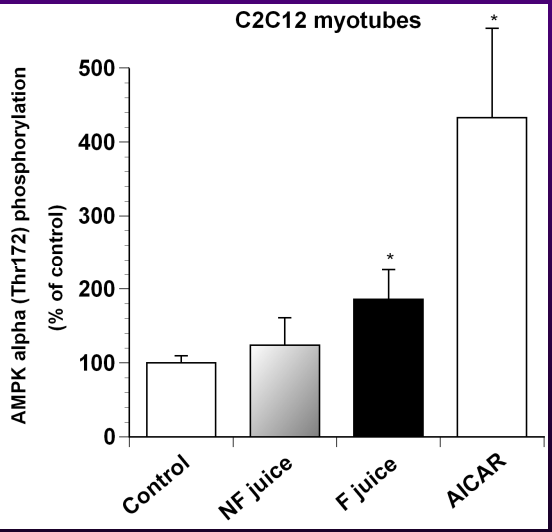
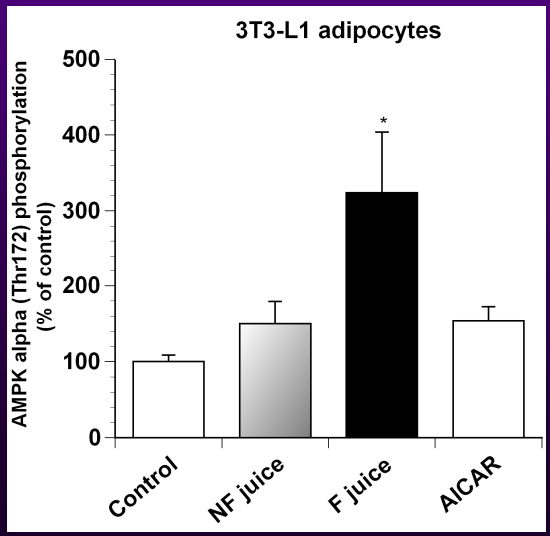
Activation of AMPK-α







Summary

Transformation by fermentation with *Serratia vaccinium* bacterium confers anti-diabetic activities to blueberry juice:

- **Increasing glucose uptake in muscle cells and adipocytes**
- **Activation of AMPK-α as mechanism of action**
- **Inhibition of adipocyte differentiation**

Conclusion

Fermented bluebery juice should be useful against the metabolic syndrome!

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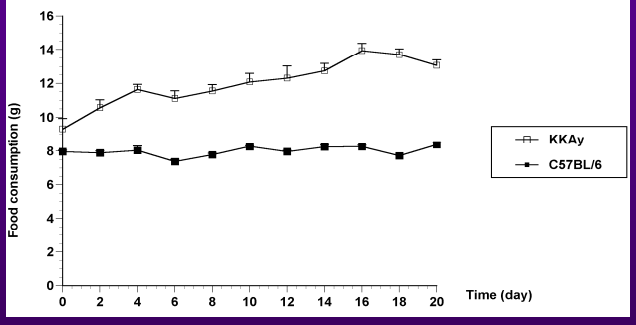
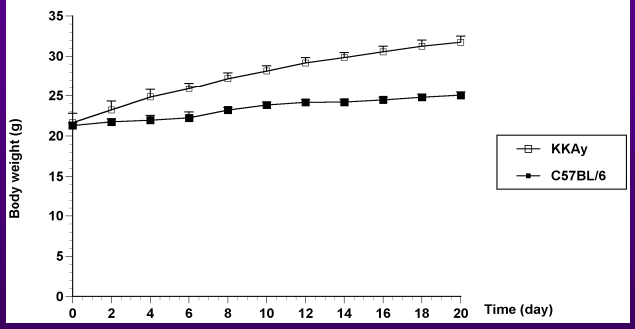
KKAy: genetically fat mouse model of Type II diabetes

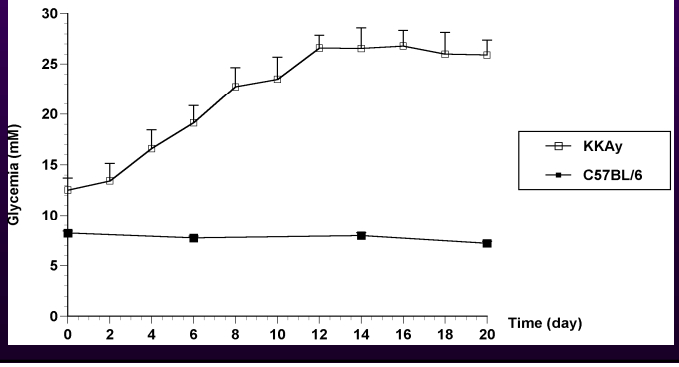
Food Consumption



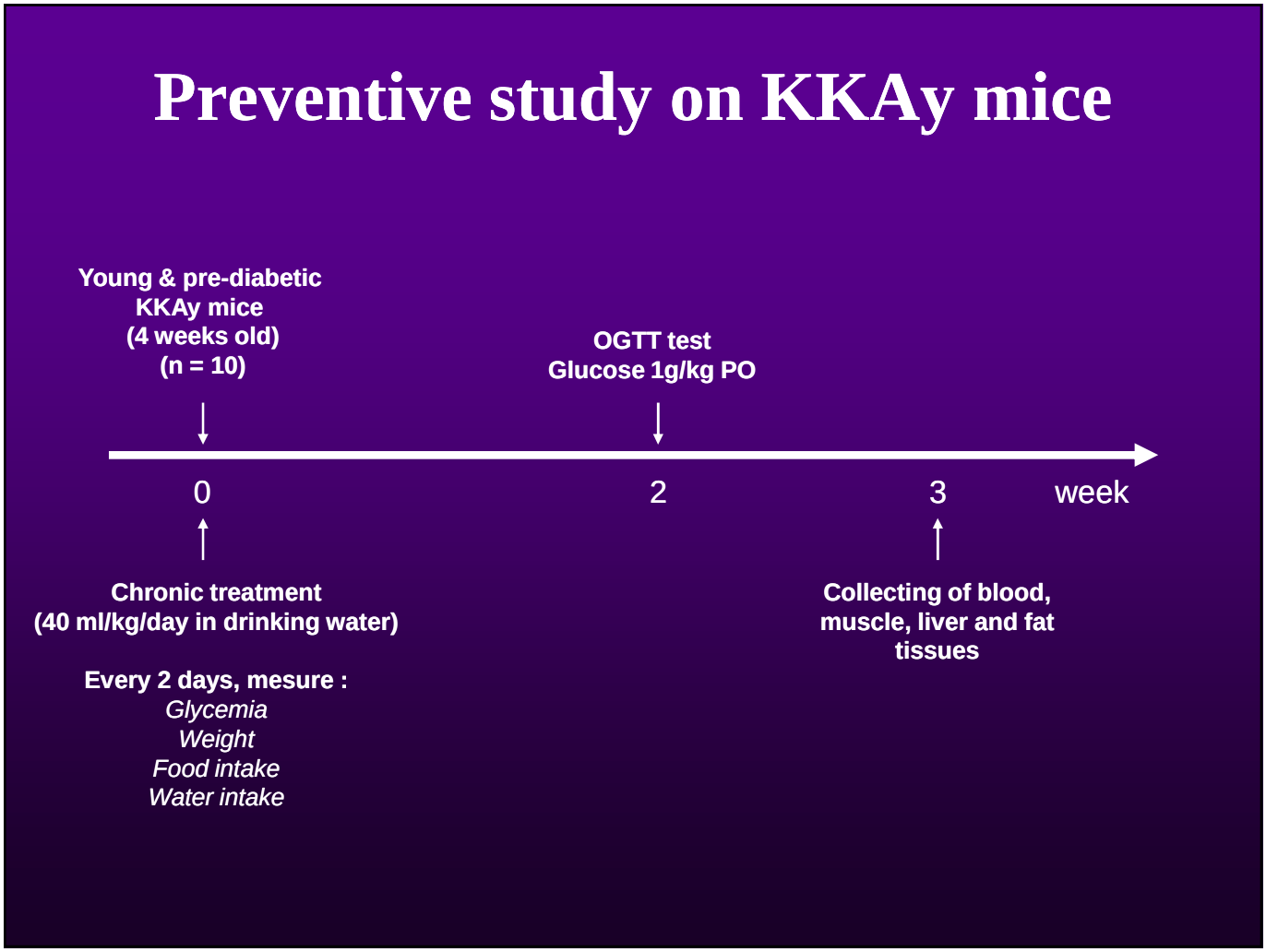
Body Weight



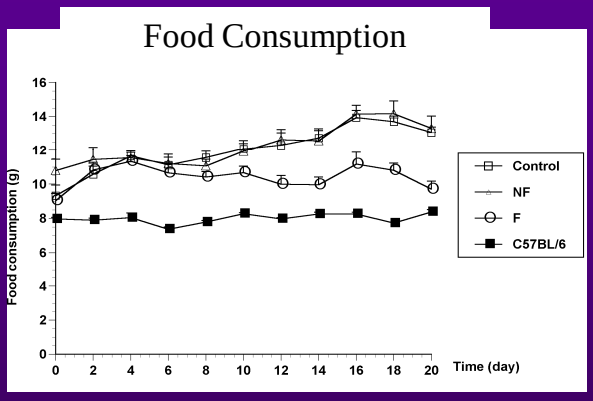
Blood Glucose

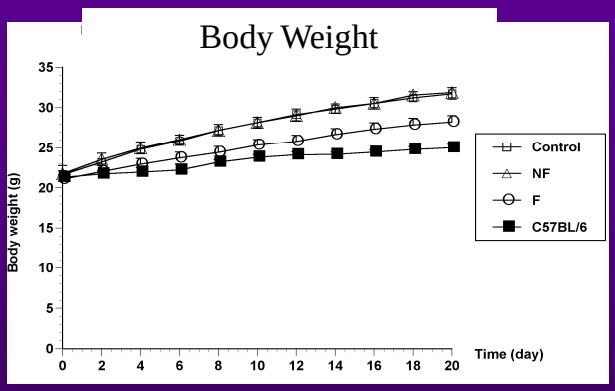


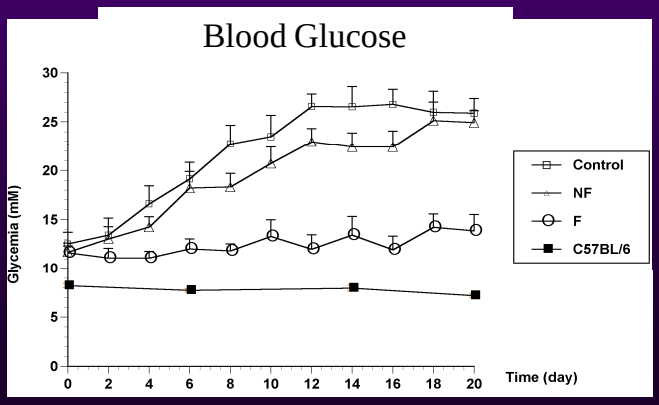
Preventive study on KKAy mice



Preventive effect of fermented blueberry juice

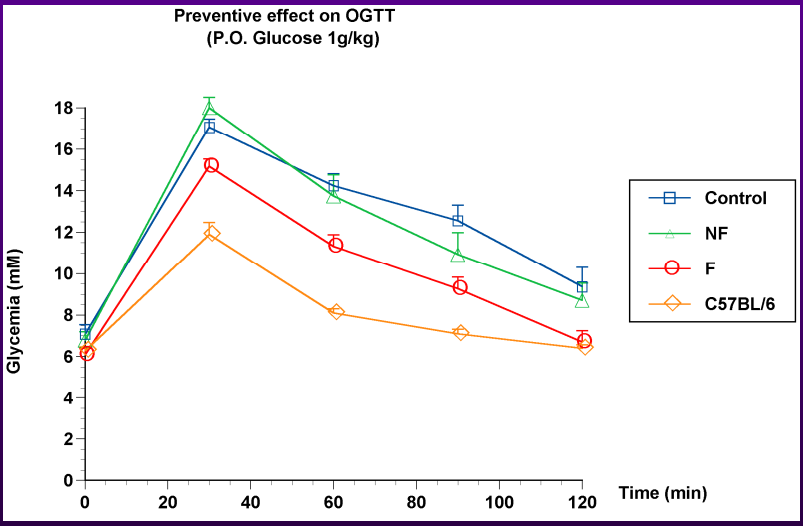


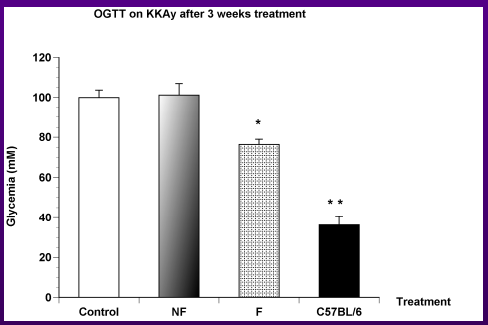




Vuong, et al. *Int J Obes* (2009) 33:1166-1173

Preventive effect of fermented blueberry juice





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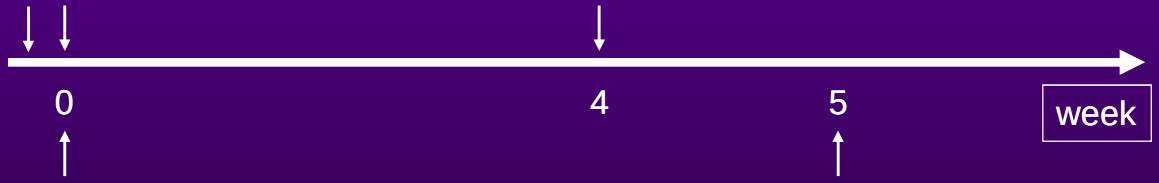
Treatment study on KKAy mice

Diabetic KKAy mice
(Glycemia : 22-30 mM)
(6-8 weeks old (n = 7)

Acute test
(5ml/kg PO)

OGTT test
Glucose 1g/kg PO

OGTT test
Glucose 1g/kg PO

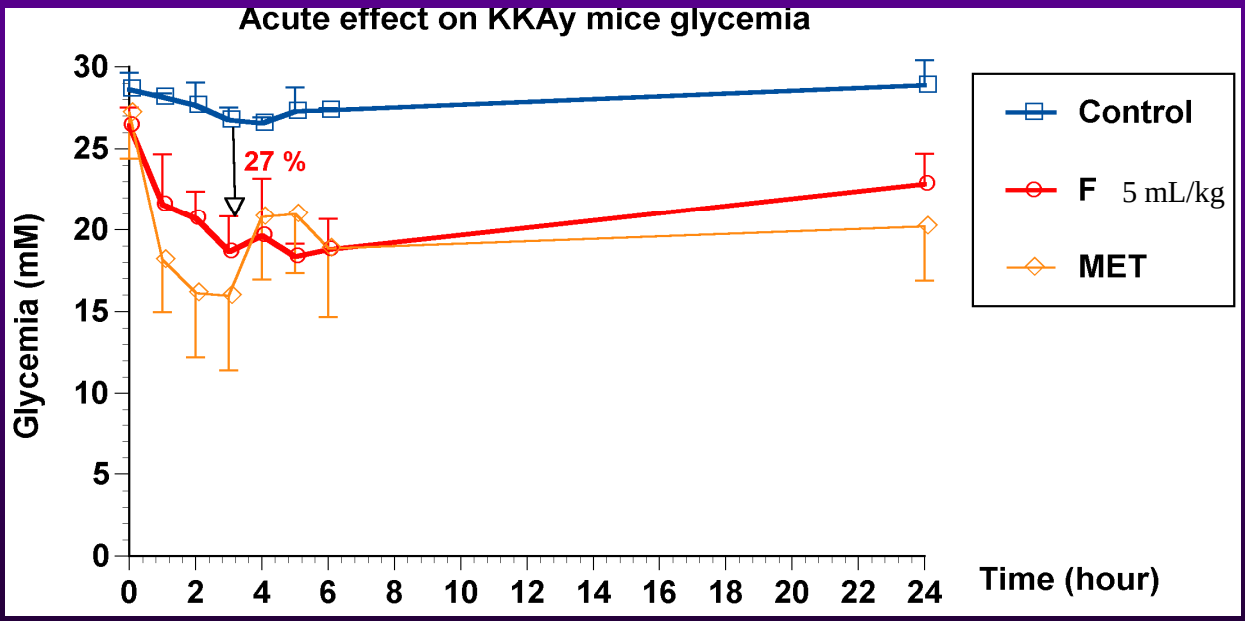


Chronic treatment
(80ml/kg/day
in drinking water)

Daily mesure :
Glycemia
Weight
Food intake
Water intake

Collecting of blood, muscle, liver and fat tissus

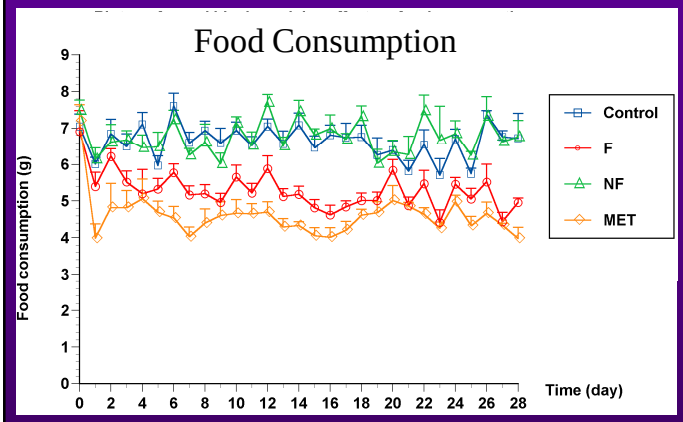
Acute antihyperglycemic effect in KKAy mice

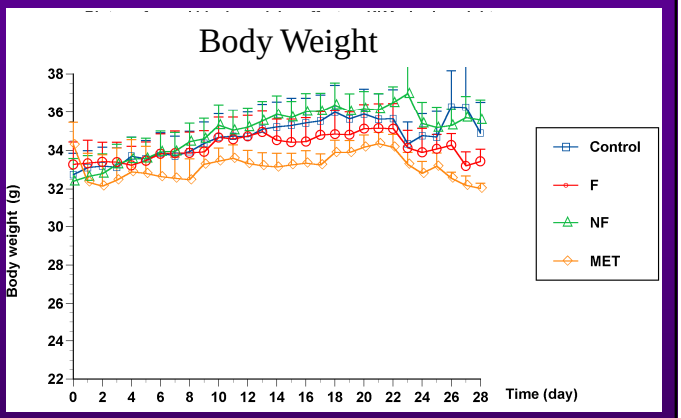


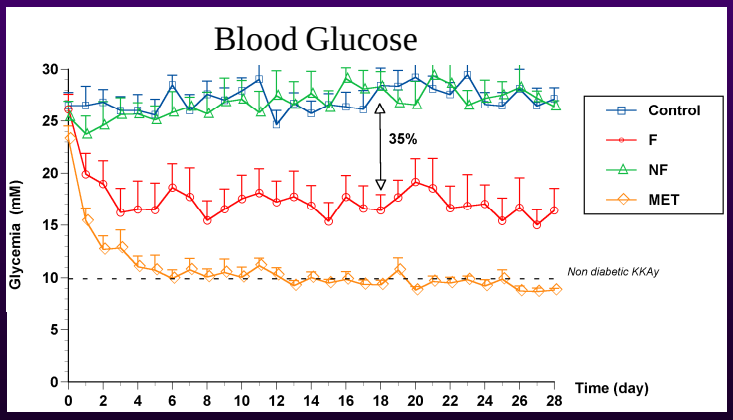
(MET = Metformin : 0.283 g/kg)

Vuong, et al. *Int J Obes* (2009) 33:1166-1173

Chronic anti-hyperglycemic effect in KKAy mice







(MET = Metformin : 0.85 g/kg/day)

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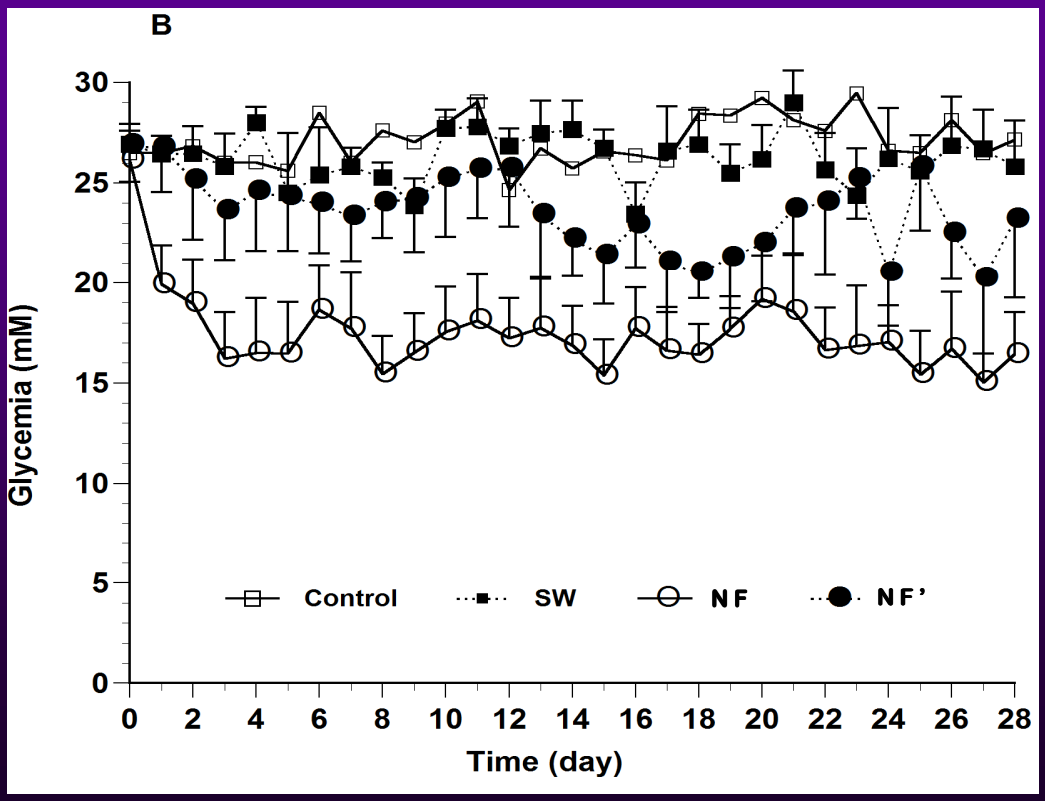
Chronic anti-hyperglycemic effect in KKAy mice
Pairfeeding experiment



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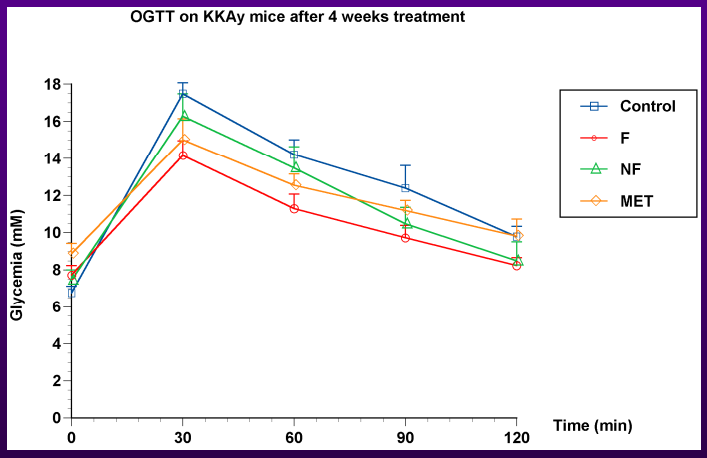
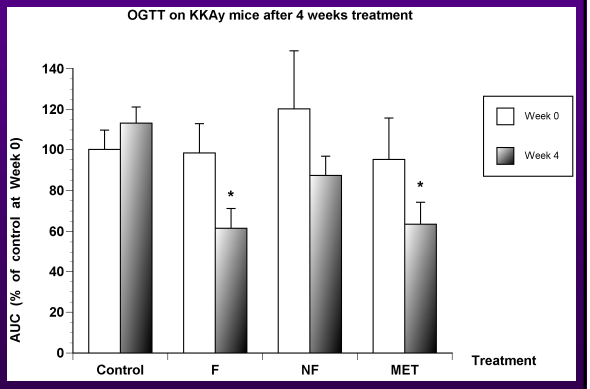
OGTT test in KKAy mice





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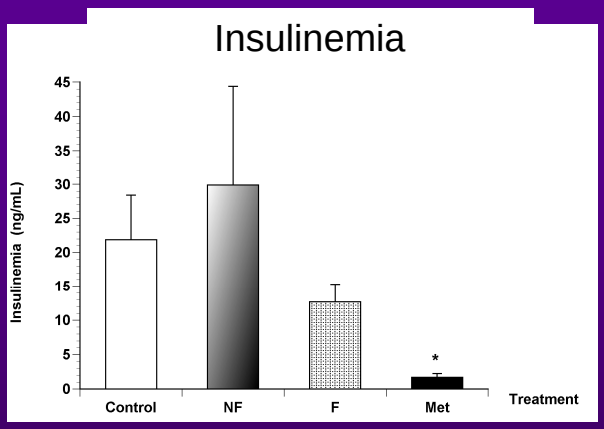
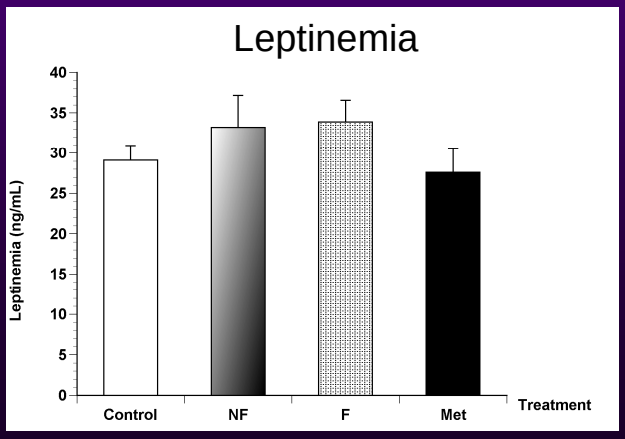
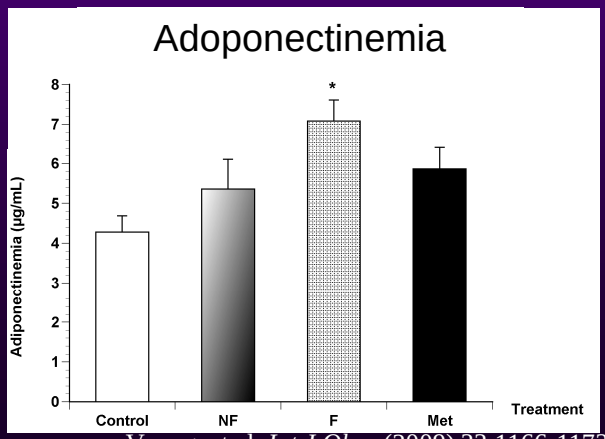
Chronic Treatment in KKAy mice







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Summary

- **Fermented blueberry juice has anti-hyperglycemic and slight weight control effects in KKAy mice (Type II diabetes)**
- **Active principles and their mechanisms of action remain to be identified**

Conclusion

- **Fermented blueberry juice represents a novel complementary therapy and a source of novel therapeutic agents against diabetes mellitus**

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Anti-hyperglycemic effect of biotransformed blueberry juice in diabetic KKAy mice.

Excerpt from U. Montreal website:

Press release January 11 2010 12:21

Top 10 Studies of 2009

8. Professor **Pierre Haddad** and PhD student **Tri Vuong**, of the Departmenet of pharmacology, win eigth place for having discovered that lowbush blueberry juice, biotransformed by a bacteria present on the skin of the fruit, constitutes a promising anti-obesitu and anti-diabetic agent. Results of this study have been presented in various media such as *Canoë, Radio-Canada, Cyberpresse, Télé-Québec, CTV, The Globe and Mail, Le Figaro, Birmingham Star* and *United Press International*


THANK YOU
Louis Martineau
Sofia →
Tri Vuong
CANADIAN
DIABETES
ASSOCIATION