

FINAL PROJECT REPORT

Project Title: Washington apple varieties for management of type 2 Diabetes

PI: Kalidas Shetty
Organization: University of Massachusetts at Amherst
Telephone/email: 413-545-1022/kalidas@foodsci.umass.edu
Address: Chenoweth Building, Department of Food Science
Address 2: 100 Holdsworth Way
State/Zip: Amherst, MA 01003

Co-PI (2): Duane W. Greene
Organization: University of Massachusetts at Amherst
Telephone/email: 413-545-5219/dgreene@pssci.umass.edu
Address: Bowditch Hall
Address 2: Department of Plant. Soil & Insect Sciences
State/Zip: Amherst, MA 01003

COOPERATORS: None

Other funding Sources: None

Total Project Funding: Year 1: \$ 45,000 Year 2: \$ 47,850

Budget History:

Item	2008	2009
Salaries (50% Research Associate)	20,000	21,000
Benefits 35% of Salary	7,000	7,350
Wages	NA	NA
Benefits	NA	NA
Equipment (HPLC Columns & Maintenance)	3,500	4,000
Supplies (Reagents and Enzymes)	7,500	8,000
Travel (Part of Travel Support for Conferences and Meetings to Present Research from this Project)	NA	NA
Miscellaneous (Apple orchard needs & preparation of materials & management for enzyme studies)	6,000	6,500
WFTRC (Fruit Samples & UPS)	1,000	1,000
Total	\$ 45,000	\$ 47,850

RECAP ORIGINAL OBJECTIVES:

- 1) To evaluate the health benefits of major fresh varieties of apples grown in Washington State for combating early stages of Type 2 diabetes and to better advance a fruit and vegetable rich healthy diet based on this information (Year 1).
- 2) To evaluate the health benefits of post-harvest stored apples under CA and 1-MCP conditions of important varieties grown in Washington State to determine if the potential evaluated under Objective 1 for combating early stages of Type 2 diabetes is maintained during storage (Year 2).

SIGNIFICANT FINDINGS:

The rationale for this study is to better biochemically define the well-known health benefits of apple that have been attributed in part to their polyphenolic metabolite content and related antioxidant capacity. The consumption of apple could provide health benefits by lowering the risk for chronic diseases such as metabolic syndrome diseases including type 2 diabetes. This chronic diet-linked metabolic disease affects 25 million Americans and 205 million globally and is projected to increase to 400 million globally by 2030. The biochemical objectives of this study are to investigate the **phenolic-linked anti-hyperglycemia** (Figure 1) **bioactive factors in apple** varieties.

Overall, whole apple consumption including peel and pulp has more complete health benefits potential relevant for dietary support for potentially managing early stage type 2 diabetes and its complications. These cannot be obtained from apple juice or other common fruits such as banana and orange which are devoid of skin component unlike whole apple.

The results of this WTFRC study clearly provide the biochemical rationale for clinical studies for integration of whole apple consumption in community food systems for managing early stages of type 2 diabetes when diet and exercise can counter this chronic metabolic syndrome disease. This is now being written for 2 community food systems projects to NIH, one submitted (NIH Pioneer Project) and is pending and a second NIH Proposal to study the effect 2 apples a day and exercise on managing hyperglycemia will be submitted in 2010.

Ten different apple varieties were analyzed in relation to peel and pulp fractions of each variety separately and extracted in distilled water and 12 % ethanol. These extracts were analyzed using *in vitro* biochemical and critical enzyme (alpha-amylase and alpha-glucosidase the target of commercial anti-diabetes drug acarabose-Figure1) analysis in the context of relevance and benefits for managing early stages of type 2 diabetes.

- 1) **Peel** sample was shown to have **higher total soluble phenolic content** and related antioxidant activity than pulp sample (Figures 2 & 3). These bioactivities are potentially important to combat cellular oxidation reactions that are high during hyperglycemia stages of type 2 diabetes.
- 2) **Overall all apples have a good baseline phenolic content and Honeycrisp and Delicious varieties have the highest total phenolic content (>800 ug/g FW)** this is correlated well to high (>70%) antioxidant activity (Figures 2 & 3).
- 3) All 10 varieties evaluated showed **moderate to high (>70%) α -amylase inhibitory activity in the pulp but low bioactivity in the peel** (Figure 4) which is sufficient to potentially control breakdown of starch but not strongly inhibit as the drug acarabose (which results in undigested starch that may cause diarrhea).
- 4) **All 10 varieties evaluated showed moderate to higher baseline alpha-glucosidase activity (also target of drug acarabose), which is significant and 4 varieties Honeycrisp, Jonagold, Golden Delicious and Red Delicious have marginally higher bioactivity than other varieties.** All varieties show good dose dependent response (Figure 5), which is very important in any further food and clinical study design and optimization.

- 5) This study provides the biochemical rationale that if whole apple (peel and pulp) is consumed with a soluble carbohydrate (high glycemic index) diet it has the potential to slow digestive process and reduce degradation of starch (alpha amylase inhibition) or sugar and slow down glucose absorption (alpha-glucosidase inhibition) and therefore slow accumulation of high levels of glucose in the blood that can otherwise result in the condition of hyperglycemia relevant to increased type 2 diabetes (Figure 1 below for **summary of mechanism of action**).
- 6) **For complete bioactive benefits whole apple consumption (peel and pulp) is superior and deliver hyperglycemia managing benefits and antioxidant benefits and bioactive functions is retained in long term stored grocery store apples treated with 1-MCP (Figure 6 & 7) and more studies are on-going on how 1-MCP treated apple phenolic-linked antioxidants and alpha-glucosidase and alpha-amylase inhibitory activities change every 2 months following 1-MCP treatment (study will be completed in June 2010).**
- 7) Some of alpha-amylase inhibitory (resistance to starch breakdown) potential may be due to insoluble polysaccharide and oligosaccharide (fiber) fractions of apple pulp and similar to standard drugs (acarabose) with **whole apple likely having potential of resulting in less side effects of undigested starch than drugs.**
- 8) The **additional benefits of high phenolic-linked antioxidant activity in the apple peel** has potential to contribute to the reduction of microvascular complications of late stages of type 2 diabetes such as wound healing, macular degeneration and kidney dysfunction.
- 9) **Quercetin (Table 1) and related flavanoids** are important phenolics from apple peel that are linked to bioactive benefits. However, overall profile of phenolics and fiber are likely more important. **A 200 gram apple with peel can provide between 2 mg to 8 mg quercetin.**
- 10) The **bioactive benefits are clear based on *in vitro* biochemical and enzyme assays** and this study provides the strong biochemical rationale for consumption of whole apples to further community food systems needs & clinical studies to meet 2-3 servings per day of US per capita fruit and vegetable consumption will be targeted. **A single 200 gram whole apple can provide up to 40 mg to 50 mg of total soluble phenolics.**

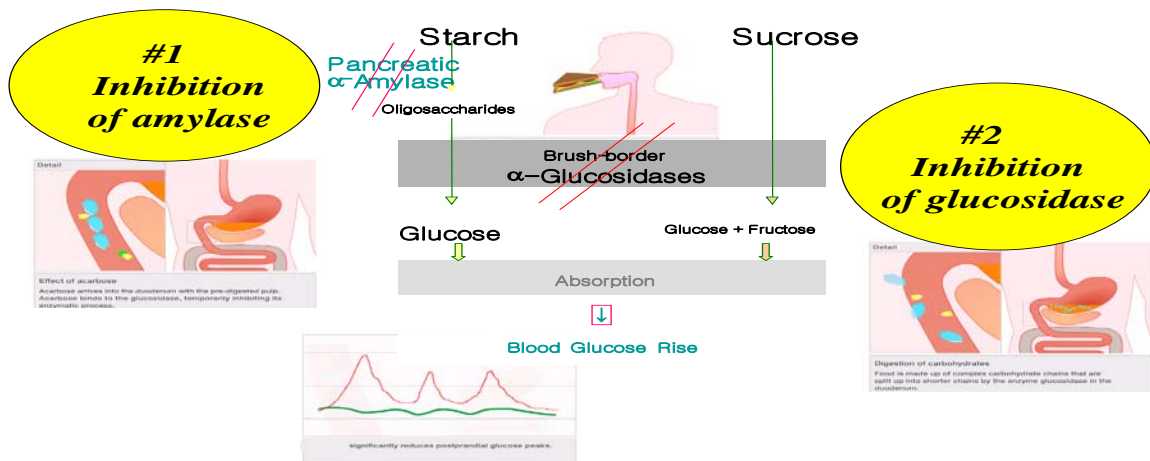


Figure 1: How alpha-amylase and alpha-glucosidase from apple work? Apple polyphenolics and especially from skin (peels) target these same enzymes as the drug acarabose which inhibits both enzymes strongly and therefore has side effects. Whole apples can deliver strong inhibition of alpha-glucosidase and milder inhibition of alpha-amylase which is preferred. The number of whole apples per day (2-3) and what stages of early stage of type 2 diabetes and combination of exercise will be determined by further studies proposed to NIH.

RESULTS & DISCUSSION:

Year 1: Objective 1: Apples were harvested from August 28, 2008 (Ginger Gold) to October 23, 2008 (Braeburn) at the University of Massachusetts Orchards in Belchertown, MA under supervision of Dr. Duane Greene based on standard harvest index standards (data available) based fruit weight, red color, circumference, firmness, starch, soluble solids and percent water core.

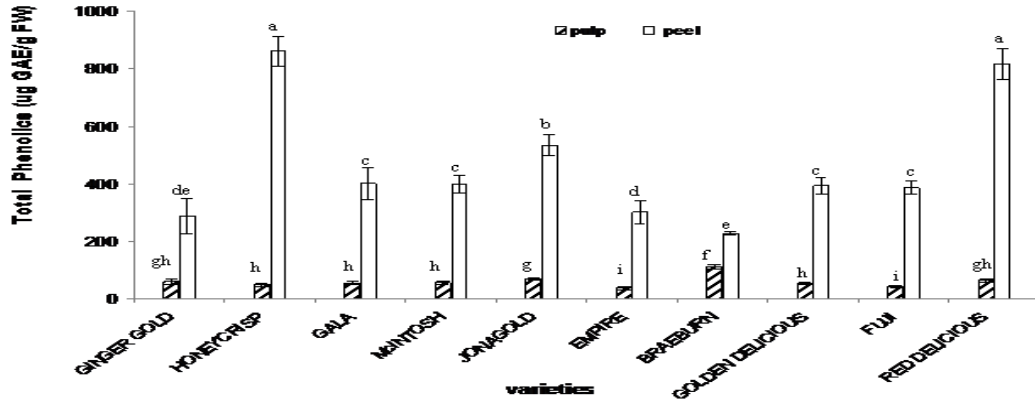


Figure 2: Total soluble phenolic content of freshly harvested apple varieties

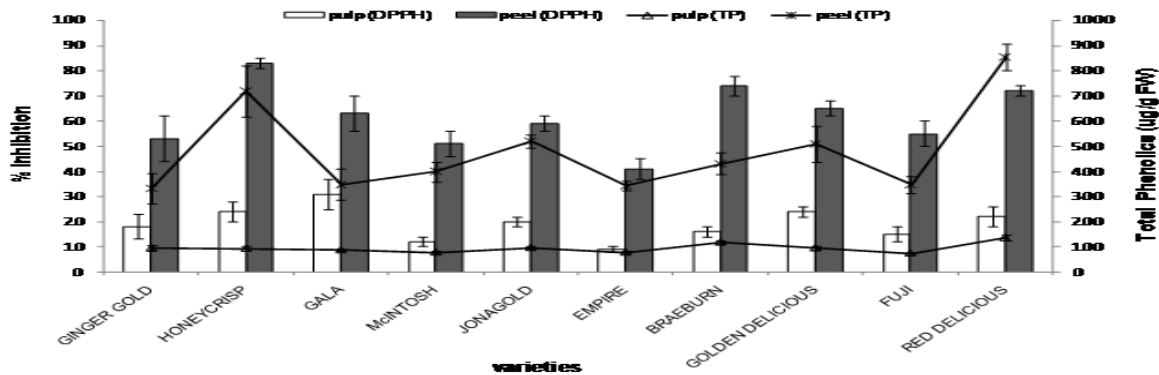


Figure 3: Total Phenolics & Free radical scavenging-linked antioxidant activity of freshly harvested apple varieties

The Peel samples have higher total soluble phenolic content and related antioxidant activity than pulp sample (Figures 2 & 3). Among these Honeycrisp and Delicious varieties have the highest total phenolic content (>800 ug/g FW) this is correlated well to high (>70%) antioxidant activity (Figures 2 & 3). **Therefore for health benefits derived from phenolics whole apples with peel would be superior.** This is particularly relevant for microvascular complications of type 2 diabetes due to cellular oxidative breakdown such as wound healing, macular degeneration and improved kidney function. In Year 2 when compared to long term stored store varieties the total phenolic content were not only maintained **but in many varieties the total phenolic content and antioxidant activity was enhanced with long term 1-MCP treated storage (Figure 6). This is significant.**

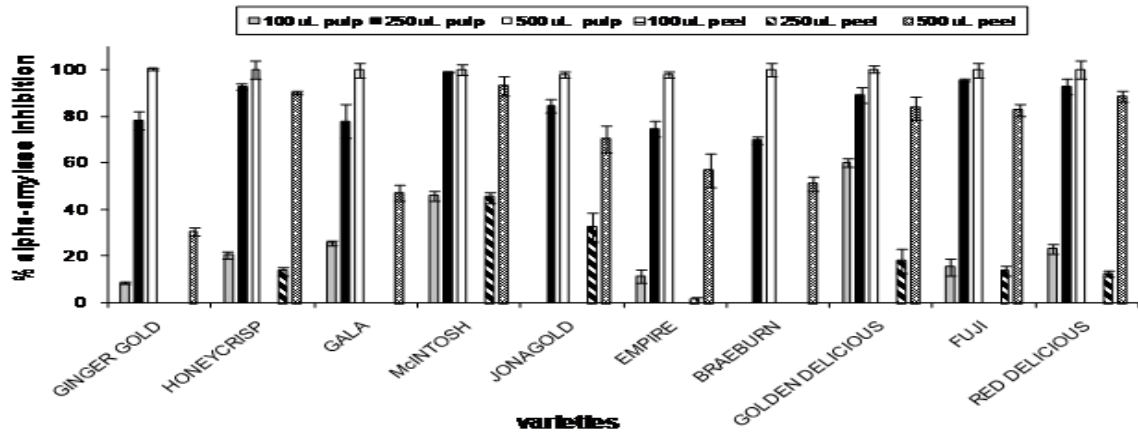


Figure 4: Alpha-amylase inhibitory activity of freshly harvested apple varieties

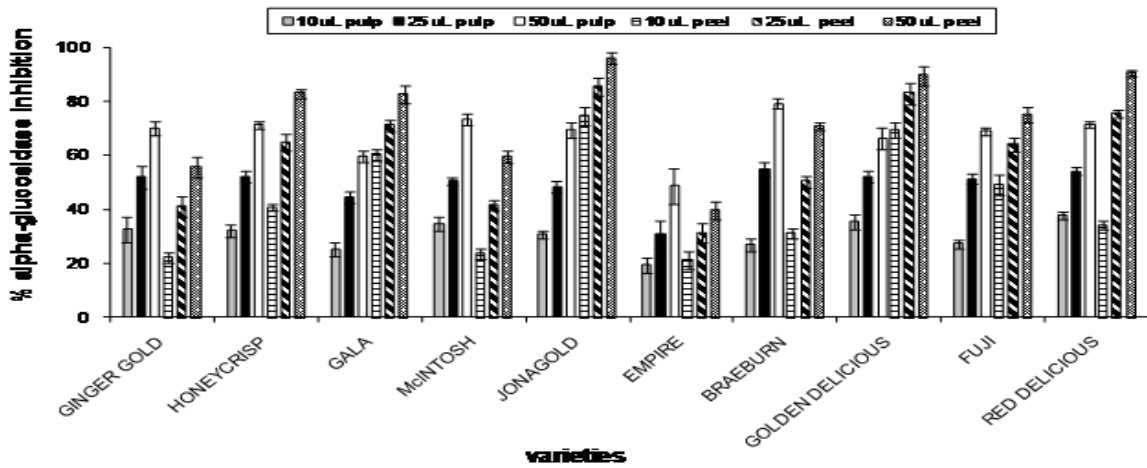


Figure 5: Alpha-Glucosidase inhibitory activity of freshly harvested apple varieties

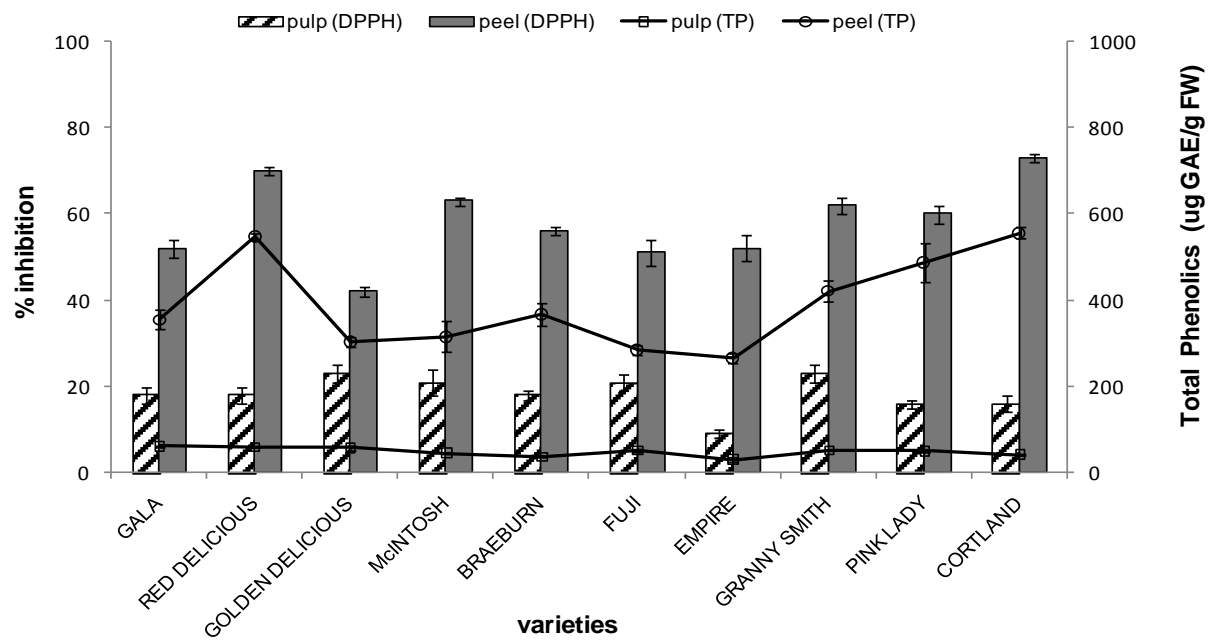


Figure 6: Total phenolics and Antioxidant activity of long term stored apples from Grocery stores (**Levels are maintained and in several cases increased with post-harvest storage**)

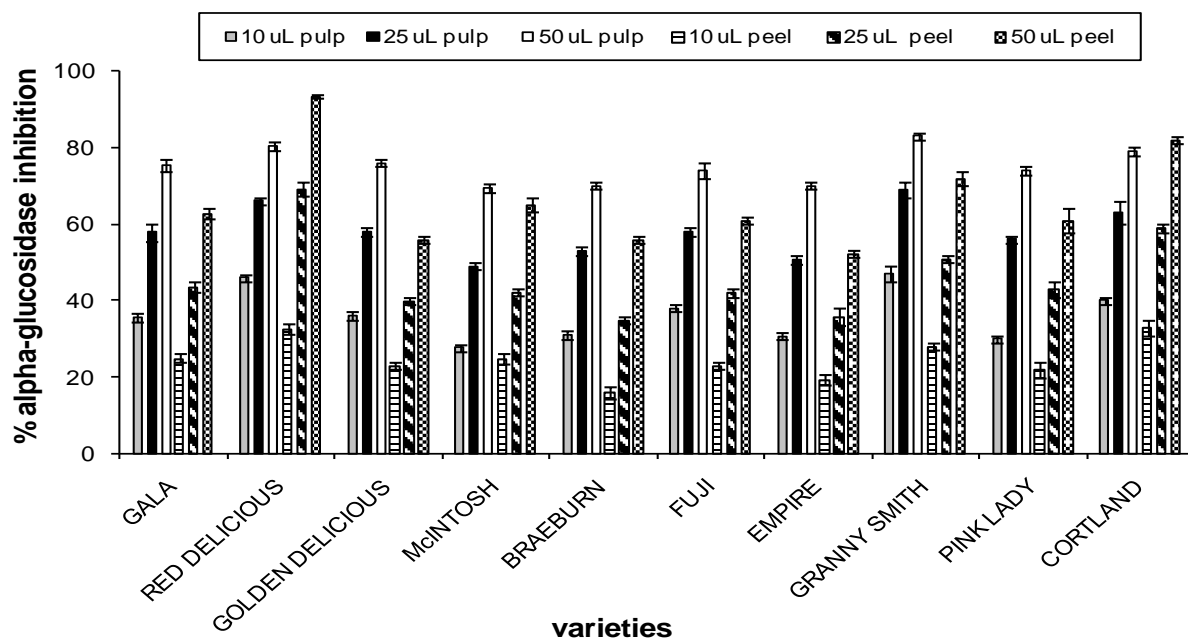


Figure 7: Long term stored grocery store varieties maintain and in some cases increase the alpha-glucosidase bioactive function in proportion to total phenolics and antioxidant activity

Table 1: Quercetin Content as Determined by HPLC: units: micrograms/gram peel)

	<u>Water Extracts of Peel</u>	<u>Ethanol Extracts of Peel</u>
Ginger gold	128 ± 5	142 ± 3
Honeycrisp	144 ± 3	554 ± 5
Gala	93 ± 2	68 ± 1
McIntosh	101 ± 2	534 ± 6
Jonagold	286 ± 5	213 ± 2
Empire	41 ± 1	327 ± 5
Braeburn	390 ± 3	419 ± 6
Golden delicious	84 ± 3	119 ± 3
Fuji	136 ± 2	74 ± 2
Red delicious	133 ± 3	171 ± 3

The peel samples have higher diabetes relevant alpha-glucosidase inhibitory activity, which the target of the current pharmaceutical acarabose for this early stages type 2 diabetes target (Figure 1 & 5). All varieties have good baseline and dose dependent activity and some varieties (Honey crisp, Jonagold, Golden Delicious and Red Delicious) are consistently a little superior. Among store brought long term stored (1-MCP treated Grocery store apples) varieties the baseline activity is maintained and Red Delicious again is slightly superior.

Discussions:

- 1) Whole apple have high phenolic content, related antioxidant activity and good dose dependent alpha-glucosidase inhibitory activity. Therefore there is merit in including 2-3 apples a day to increase daily intake of fruits and vegetables to increase to 5-6 servings needed to manage current fast growing epidemic of metabolic syndrome disease.
- 2) Whole apple with peel (skin) and pulp with reduced soluble sugar have the best potential.
- 3) Long term stored apples can retain the bioactive benefits and in case of phenolics-linked antioxidant activity the benefits increase.
- 4) High quercetin varieties (Table 1) are significant in the context of using apples and exercise to manage early type 2 diabetes and in the context of general endurance where quercetin is being targeted as an important biomolecule for sports and general endurance. In this case also the whole apple with peel is very important.

Note: The results of 2009 harvest of both fresh harvest and 1-MCP treated stored apples comparing to 2008 results is still on-going and will be submitted by end of Spring when the analysis will be completed. Here only select varieties were selected to confirm previous fresh harvest study and to 1-MCP treatments and whether this changes during every two months of storage. The results of long term stored grocery store apples (Figures 6 & 7) clearly indicate no changes in bioactive functions and in many cases increase in phenolic and antioxidant bioactive functions. This increase will be followed in the current 1-MCP studies that are on-going and results available as a publication format in early June 2010. The cultivars that are being investigated are: Gala, Honeycrisp, Golden Delicious, Red Delicious, Jonagold and Braeburn that cover lower to higher phenolic ranges.

Significance to apple industry and economic benefits:

Two important 2009 National and Global Reports clearly indicate the benefits of this research and benefits to the apple industry.

1) The States Indicator Reports on Fruits and Vegetables

<http://www.fruitsandveggiesmatter.gov/downloads/StateIndicatorReport2009.pdf>

2) The rapid growth of global & US type 2 diabetes epidemic

<http://www.idf.org/international-diabetes-federation>

<http://www.cdc.gov/diabetes/>

Less than 30% of the US population eats the recommended 2 or more servings of fruit a day and 3 or more servings of vegetable a day. This falls well short of 5-6 servings per day of fruits and vegetable for the population as a whole and even 9-10 servings per day recommended by some for serious management of chronic diseases and chronic diet-linked metabolic syndrome diseases.

One of the consequences of modern refined diet and reduced consumption of fruits and vegetables is the rapid increase in metabolic syndrome diseases including type 2 diabetes which is now affecting 25 million of the US population. This is projected to affect 10% of the population in this decade with minority communities being affected up to 15%-20% of their population. Diet and exercise can prevent and help manage these conditions and this requires more precise definition and biochemical analysis of benefits of fruits and vegetables based cellular and enzyme based structure-function rationale. This research has clearly provided this strong foundation.

Apples showed relatively high levels of total phenolics and antioxidant capacity comparable to those of oranges and their phenolics and antioxidants contribution is the second highest in the American diet (Barbosa et al., submitted to *J. Medicinal Food*; Chun et al., 2005; *J Sci Food Agric* 85:1715–1724). However, only whole apples (compared to bananas and oranges) have with better defined structure-function characterization of skin and peels as undertaken in this study (Barbosa et al., submitted to *J. Medicinal Food*), to rapidly help increase US per person intake of fruits and vegetables. If we can increase the average US annual apple production by **100 Billion apples per year** it can increase overall intake by just 1 serving per day per person. This is the most effective antidote for chronic diet and environmental-linked diseases combined with exercise. Therefore not only production of fresh apples has to increase but quality of these apple varieties for chronic disease management has to be better characterized.

Clearly for maximum health benefits in the context of better diet for prevention and early stages of type 2 diabetes management, **whole apple including pulp and peel has best potential when compared to other common fruits such as banana and orange.** These bioactives from whole apple have the potential to influence positively multiple physiological pathways from soluble carbohydrate utilization control to oxidative breakdown of this chronic disease. This biochemical rationale can be basis of better fruit and vegetable diet that could include 1-3 apples per day. The results from these current studies can provide the **key biochemical rationale** for future clinical studies that can be the basis for how many apples/day may be relevant and for what stages of type 2 diabetes such as pre-diabetes, when diet and exercise are most effective. Therefore better defining the biochemical basis of health benefits of apple contributes to enhanced fresh apple consumption globally. Further, based on biochemical rationale from this study and further clinical studies as the next step could show that **whole apple with peel and pulp is potentially superior** to juice from pulp, where high phenolic skin and fiber are discarded. **One 200 gram apple a day can provide 40 mg to 50 mg of total phenolics** which is more than the average total soluble phenolic intake per day in the United States. **Apple industry is the only fruit industry in the United States that has the best prospects to rapidly increase the phenolic-enriched fruit and vegetable consumption needed for managing chronic diseases. It can also be integrated into Community Foods Systems and winter needs.**

Publications from this study:

Book Chapter:

Shetty, K., Adyanthaya, I., Kwon, Y-I., Apostolidis, E., Min, B-J., Dawson, P (2008). Postharvest enhancement of phenolic phytochemicals in apples for preservation and health benefits. In: **Postharvest Biology and Technology of Fruits, Vegetables and Flowers** (Paliyath G, Murr D, Handa AK, Lurie S {eds}) 2008, Chapter 16, Pages 341-371. Wiley-Blackwell Publishing, Ames, Iowa, USA.

Manuscript 1:

Barbosa A.C.L., Pinto,M.D.S., Sarkar,D., Ankolekar, C., Greene D.and Shetty K. (2010) Varietal Influences on anti-hyperglycemia properties of freshly harvested apples using *in vitro* assays models. Submitted for Publication to **Journal of Medicinal Food (Pre-publication manuscript available on request to WTFRC)**.

Manuscript 2:

Barbosa A.C.L., Pinto,M.D.S., Sarkar,D., Ankolekar, C., Greene D.and Shetty K. (2010) Influence of varietal and pH variation on anti-Hyperglycemia and anti-hypertension properties of long-term stored apples using *in vitro* assay models. In preparation for Publication to **Journal of Food Biochemistry (Pre-publication manuscript available on request to WTFRC)**.

Manuscript 3:

Sarkar,D., Barbosa A.C.L., Pinto,M.D.S., Ankolekar, C., Greene D.and Shetty K.. (2010) Phenolic-linked antioxidant and anti-diabetes potential of 1-MCP treated apples during post-harvest storage. On-going research and in preparation for Publication to **Journal of Agricultural and Food Chemistry (Data and pre-publication manuscript will be available in early June 2010)**.

Current and Future Proposals from this study:

USDA-Health and Wellness Special Grant:

University of Massachusetts Amherst, Department of Food Science has just been awarded (April 2010 to March 2012) \$ 525,000 for Health and Wellness Research. Out of this \$ 40,000 has been awarded to PI, Kalidas Shetty to advance inclusion of apples and cucurbits as a part of community food systems for management of metabolic syndrome diseases, including type 2 diabetes. The goal is to enhance inclusion in daily diet of high phenolic fruits (2 apples a day) and vegetables (2 cucurbits a day) with anti-diabetic and anti-hypertension bioactives in several communities of Hampden and Hampshire counties in Western Massachusetts, Based on this community food systems integration a framework and community trust will be built for subsequent clinical studies in collaboration with Baystate Medical Center.

NIH Pioneer:

PI, Kalidas Shetty has submitted a proposal to NIH a 5 year proposal for \$ 2.5 million over 5 years to develop “Community Food Systems to Manage Type 2 Diabetes” based on structure-function basis for designing and making available fresh fruits and vegetables. This project provides the framework to develop locally grown fresh foods, high school fresh fruit and vegetable program, collaboration for clinical studies with local hospitals and national network of fresh fruit and vegetables based on bioactive properties for combating chronic metabolic syndrome diseases. In this project type 2 diabetes relevant 2 apples a day is an important part of strategy to increase fruit and vegetable intake to more than 5 serving a day over the next 5-7 years.

NIH-NIDDK

PI, Kalidas Shetty has joined with 2 Exercise scientists to study the interaction of endocrine and stress modulating phenolics and exercise for managing type 2 diabetes and also general endurance in athletes for effective recovery after exercise. In this regard high quercetin apples (Table 1) and few other mono-phenolics in rare apples in Urals, Kazakhstan and some old varieties in New England have high potential. Based on preliminary studies a NIH proposal will be developed in 2010-2011 for use of high phenolic Apples for type 2 diabetes management in combination with exercise and endurance and stress recovery in athletes. Apple, Pear and Cherry with the right varieties have potential for athletic, stress and endurance performance.

NIH-Fogarty International Program

This project proposal in planning stages in Pskov Agricultural College in Russia and Agricultural Ministry in Kazakhstan focuses on developing strategies to maintain and manage apple diversity in Russia and Kazakhstan and especially high phenolic extreme weather tolerating varieties. This diversity project has 2 objectives 1) to preserve rare varieties that have best potential to withstand extreme weather changes based on climate change 2) high phenolic dietary modulators that are relevant for managing metabolic syndrome diseases and daily endurance and stress management.

NSF-Community and Population Ecology Program

Based on the research on understanding type 2 diabetes relevant phenolics, there are specific varieties that have enhanced profiles of phenolics with better post-harvest preservation using 1-MCP (Figure 6) and further have high levels of phenolics like genistein released only in ethanol extracts (Table 1). This provides clues about specific structural linkages of phenolics in peels (skins) dependent on varieties. We will be exploring how specific beneficial bacterial such as lactic acid bacteria change and interact with apple skin and modulate phenolics. This has implications for post-harvest fitness of apples and preservation of apples and use of lower cost fallen apples in community food systems projects. The most exciting part of this project is that there is potential for producing antimicrobials from beneficial bacteria and phenolics from plants to enhance agronomic stages of apples and Rosaceae in terms of stress and disease management. Interactions of beneficial bacteria with probiotic function is not only important for animal function but is being observed as being important for plant functions including stress and reproductive (flowering and fruiting) function. This provides new approaches for organic based production of apples based on stress biology linked to plant phenolic and beneficial bacterial interactions.

NSF-Physiology and Structure

This is a basic research proposal in planning stage by PI and Co-PI and will examine how beneficial bacterial modulate phenolic-linked antioxidant enzyme response in post-harvest preserved apples with and without 1-MCP. This research is based on our recent publication on role of redox biology in post-harvest preservation of apples (Adyanthaya, I., Kwon, Y-I., Apostolidis, E. and Shetty, K. (2009) Apple post-harvest preservation is linked to phenolics and SOD activity. *J. Food Biochemistry*, 33: 535-556). Understanding from this research will advance new strategies for post-harvest preservation of apples and natural and probiotics to control the right post-harvest biochemistry.

EXECUTIVE SUMMARY:

One 200 gram apple a day can provide an average of 40 mg to 50 mg of total soluble phenolics which is more than the average total soluble phenolic intake per person per day in the United States compared to Okinawa, Japan (longest living population on earth), who have 600 mg to 800 mg per day per person. Therefore US Apple industry is the only fruit industry in the United States that has the best prospects to rapidly increase the soluble phenolic-enriched fresh fruit and vegetable consumption needed for preventing and managing diet-linked chronic diseases. This is especially essential for preventing and managing metabolic syndrome disease such as early stages of type 2 diabetes and general chronic disease affiliations linked to obesity. Among various regions Pacific Northwest must be targeted for high value fresh apples with health benefits. Increased fresh apple consumption is one of the most cost effective ways to increase fruit and vegetable consumption from the current levels where more than 70% of the US population DO NOT consume the recommended 2 or more servings of fruit a day and only less than 30% do.

This study indicates that whole apple in general and some varieties like Honeycrisp and Delicious have high phenolics and free radical scavenging-linked antioxidant activity in the peel. Several varieties have high activity profile of α -glucosidase inhibition (enzyme target of anti-diabetes pharmaceutical drugs for managing early stages of type 2 diabetes) and moderate to high α -amylase inhibition in the pulp. This indicates that a **complete whole fruit** offers the best potential for good postprandial blood glucose management linked to hyperglycemia associated with type 2 diabetes and its oxidative stress complications without the common side-effects associated with very high α -amylase inhibition in drugs such as acarbose. Compared to drugs, whole apple also have free radical scavenging-linked antioxidant activity which can help maintain the redox balance in susceptible cells. This study provides a strong biochemical rationale for further clinical studies to include apple as an important part of the overall diet and medicinal therapy for better management of early stages of type 2 diabetes and its complications when better diet and exercise are effective. This also helps basis for breeding of better apple varieties for better health and increase overall fruit and vegetable consumption in the United States.

Overall whole apple with pulp and peel have the best combined bioactives for maximum potential for use in diets for early stages of managing type 2 diabetes and its complications. Bioactive compounds beyond phenolics in the fiber fractions are also important and need to be investigated as part of community food systems project and clinical studies. Based on the *in vitro* biochemical rationale and results of this study, inclusion of 2 apples a day project as a part of community food systems improvement is being undertaken in specific communities in Western Massachusetts to develop a national model.

This project support from WTFRC has resulted in 1 manuscript already submitted for publication in Journal of Medicinal Food and two more in preparation (one to Journal of Food Biochemistry and another to Journal of Agricultural and Food Chemistry). In addition 1 book chapter has been published.

The foundations of this research funded by WTFRC have helped to develop new concepts and project proposals for further advancing health benefits and consumption of whole apples in the American diet. Currently a USDA grant to advance community food systems has been funded (\$ 40,000) and a major proposal (\$ 2.5 million) to NIH Pioneer program is pending. Additional proposals to federal programs at NIH and NSF are planned for 2010 and beyond with apple as the major focus. Among these proposals the relevance of apple with high phenolics for endurance and stress management in chronic diseases and athletic performance & exercise recovery is a major focus.