Introduction

The KAHEAD Project’s Plan of Work includes several objectives and activities that are specifically designed to allow the timely implementation of these objectives. Earlier correspondence has dealt with overall implementation aspects. This report builds on the latest one, submitted on May 3, 2010 and similarly pertains only to the training of Graduate Research Assistants (GRAs) funded by KAHEAD scholarships at the University of Hawaii (UH).

The reader is urged to consult the May 3 report in order to gain a total view of the progress of PhD candidates Awat Yousif and Seerwan Abdullah. Both have continued as classified graduate students at UH, and are currently enrolled as ongoing students.

This is a report of the two students’ progress up to the date of this document.
Status Report for Mr. Awat Yousif

PhD Candidate
Department of Molecular Biosciences and Biosystems Engineering (MBBE)
Area of Specialization: Animal Science

Awat has been in Hawaii since October 23, 2008; and started academic study since the Spring semester of 2009. His progress shows expected completion of course credit requirements at the end of the Fall semester, 2010. He has also undertaken research skill training with his faculty supervisor, and so is now fully prepared to start his PhD research. Excerpts of his dissertation research proposal are attached below.

Objectives

1. The objectives of this study is to determine whether myostatin is present in and expressed in the placenta of transgenic mice over-expressing a myostatin inhibitor, myostatin propeptide.
2. Evaluate the myostatin effects on placental glucose uptake associated with pregnancy.
3. Investigate the reason of placental glucose uptake change, is it belong to pups size or maternal genotype.
4. Examine effect of myostatin on some reproductive hormones and characteristics.
5. To find the correlation between placental glucose uptake and some reproduction hormones (for example Progesterone).
6. Examine changes in the levels of active form of myostatin (13 kDa) in serum after birth by Western blot to predict the role of serum myostatin in early postnatal muscle growth in the transgenic mice over-expressing a myostatin inhibitor, myostatin propeptide.

Background

Myostatin is a member of the transforming growth factor (TGF-β) - super family, known for its ability to inhibit muscle growth. It can also regulate metabolism and glucose uptake in a number of tissues (3). Myostatin knockout mice display a marked increase in muscle mass and are considerably bigger than wild-type mice at birth. The increased birth weight indicates that the fetal nutrient supply via the placenta must increase to allow the extra growth of the larger heavily muscled fetus, and this may relate to the metabolic role of myostatin in controlling glucose uptake. Therefore, myostatin may control placental size, cellular composition of the placenta, and metabolic transfer of nutrients, providing a therapeutic route to treat conditions such as placental insufficiency. Hence, we hypothesized that myostatin may be produced by the placenta and act in a paracrine manner to regulate glucose uptake (25).

Myostatin, a negative regulator of muscle growth, affects glucose uptake independent of insulin and also regulates placental glucose uptake in vitro in the human placenta. Maternal under-nutrition during early gestation has been associated with fetal programming for increased risk of metabolic disorders in late life (29). The expression and activity of myostatin is regulated by a number of proteins including the myostatin propeptide (LAP; Latency associated peptide), GASP-1 (Growth and differentiation factor-associated serum protein-1) and follistatin all of which are known to prevent the binding of myostatin to its receptor ActRIIB (28). The placenta is an important mammalian reproductive organ responsible for fetal nutrition, respiration, and waste removal. During pregnancy, an increase in the functional capacity of the placenta (i.e., the ability to exchange materials between mother and fetus) is needed, because the supply of nutrients and oxygen across the placenta must meet the increasing requirement of the developing fetus (4).
Glucose is the major fuel for energy metabolism and growth in embryogenesis, and yet the mammalian embryo is unable to manufacture this essential substrate until late in development (6), (22). Hence, the transfer of glucose from the maternal circulation to the conceptus is a crucial feature of mammalian development. The placenta plays a key role in this transfer process, which appears to be primarily a function of facilitated glucose transport down a concentration gradient from maternal to fetoplacental compartments (21, 19). The molecular basis for placental glucose transport and its regulation remain poorly understood at present. (37).

In recent years, there has been rapid progress in elucidating the molecular basis for glucose transport across cell membranes. At least five different facilitative glucose transporters (GTs 1-5, named in order of their cloning) have been characterized to date (26, 32). These integral membrane glycoproteins display significant sequence homology and appear to be members of a genetically related transporter family. The glucose transporter isoforms show unique tissue-specific patterns of distribution and may be subject to differential hormonal regulation (24). For example, GT4 is preferentially expressed in fat and muscle and is regulated by insulin (14, 10). GT2 is localized in pancreatic beta cells, hepatocytes, renal proximal tubules, and intestinal epithelium (32, 33), while GT5 appears to be expressed primarily in intestine epithelium (14). (11) suggest that GLUT 3 plays a major role in placental glucose uptake and metabolism. They show that placental GLUT 3 mRNA and protein expression can be stimulated in vivo under hyperglycemic conditions. Thus, GLUT 3 transporter isoform appears to be highly sensitive to ambient glucose levels and may play a pivotal role in the severe alterations of placental function observed in diabetic pregnancies. (36) show that glucose transporter 1 and 3 in ruminant placenta are the major routes for glucose transport across placental membranes. Only two of the 12 different isoforms of glucose transporters have been identified in placental membranes directly involved in transplacental glucose flux (37), (13), (7).

The molecular mechanisms by which environmental signals alter placental nutrient transport capacity remain unknown but may involve the imprinted Igf2 gene. This gene controls placental growth and its placental expression is down-regulated in rodents by conditions known to reduce placental size, such as nutrient restriction and glucocorticoid administration (18), (1). Disruption or deletion of Igf2 gene expression causes placental growth retardation while, conversely, Igf2 overexpression by imprint relaxation or disruption of the IGF-II clearance receptor leads to placentomegaly. Both up- and down-regulation of Igf2 gene expression alter placental phenotype and efficiency (17).

Myostatin inhibits glucose uptake in placenta cells, but follistatin (is known to block the effect of myostatin in muscle) treatment alone increased glucose uptake, while exogenous myostatin has no effect on protein level (3). Myostatin is expressed in the human placenta, and expression changes with gestation, suggesting that myostatin may have a metabolic role in the human placenta (25). There are many studies have clearly shown that myostatin inhibits glucose uptake in placental (BeWo) cells. Given that myostatin regulates nutrient uptake in other tissues, these interesting observations suggest that local placental myostatin may control placental glucose metabolism within the fetoplacental-maternal unit (3).
Activity of the placenta glucose and amino acid transport systems is influenced by gestational age and a range of environmental factors including heat stress, hypoxia under-and over-nutrition, as well as exposure to hormones, such as glucocorticoids, growth hormone and leptin (12), (30). Less is known about the regulation of the abundance of the actual transporter proteins. In rodents and sheep, placental GLUT3 mRNA and protein levels increase as gestation advances whereas GLUT1 abundance is unaffected or decreases towards term (37), (7).

The placenta produces a number of hormones including steroids, peptides, glycoproteins and eicosanoids, which are released into both the maternal and fetal circulations (15). Some of these hormones, such as progesterone and placental lactogen, have metabolic effects in the mother that favour glucose delivery to the fetus. Others like the prostaglandin (PG) F2α and E2 affect fetal endocrine function, regional blood flow and myometrial contractility, which affect placental nutrient and oxygen transfer indirectly (35). In sheep, horses and monkeys, undernutrition during late gestation increases uteroplacental production of PGF2α and PGE2 (9), (16). Progesterone inhibits insulin-induced GLUT4 translocation and glucose uptake in adipocytes (34). The actual placental capacity for glucose transfer increases between mid and late gestation and in some, but not at all, models of placental size restriction (27).

Reciprocal embryo transfer between large and small breeds has shown that growth of the placental exchange surface is controlled by both the maternal and fetal genome (2). When the genetic potential for placental growth is constrained in the thoroughbred foal by the smaller surface density of microcotyledons in the pony uterus, the chorionic villi appear to elongate, which increases the total microscopic area of feto-maternal contact in absolute terms and as a proportion of gross surface area (2). This leads to a large fetus than would be produced by a pony genotype within the pony uterus. However in pigs, the increase in fetal weight produced by transferring embryos from small breeds into the uteri of larger breeds is due to increased placental vascularity rather than weight (8).

It was observed that double muscle has varying degrees of sub fertility, higher incidence of calving difficulty, lower calf viability and increased stress susceptibility. It was observed that the height, width and area of pelvic opening were significantly smaller in the double-muscled than the normal cows. This could indicate that higher incidence of dystocia and prenatal mortality in double muscled cattle may be smaller area of pelvic opening in the double muscled cattle (5). It was also observed that double muscled cattle are more prone to more fatigue during forced exercise compared to normal cattle (20). It was also observed that large muscle mass in the double muscled cattle resulted in higher heat production during heat stress and lower capacity for heat dissipation. Double muscled cattle showed reduced fertility and were less capable of carrying to term. It was also suggest that double muscled cattle embryos have higher mortality rate (31). Gestation period for double muscle calves is longer and this resulted in higher birth weight of offspring than the normal calves.

**Investigation 1: Role of myostatin in placental glucose uptake and fertility**

This study was designed to determine whether myostatin is present in and formed by the placenta of myostatin inhibited mouse, and to evaluate the myostatin effects on placental glucose uptake during different stage of pregnancy. Earlier work (25) showed that myostatin is synthesized, released
and acts within the human placenta. It contributes to placental glucose homeostasis, because of lack information in this area especially in mice, we designed this study.

Through this study we are going to examine if any change will happen in glucose uptake rate, we want to clarify if this change because of maternal genotype or because of pups size inside the uterine. In addition to examine the effect of myostatin on some reproductive characteristics, another purpose is to find the correlation between placental glucose uptake and some reproduction hormones.

**Investigation 2: Role of serum myostatin during postnatal period**

This experiment was designed to examine changes in the levels of active form of myostatin (13 kDa) in serum after birth by Western blot to predict the role of serum myostatin in early postnatal muscle growth in the myostatin inhibited mouse. It will entail two groups of mice, **First group (Control):** 30 wild type (WT) male pups; **Second group: **30 transgenic (TG) male pups; we are going to sacrifice 5 pups from each group at postnatal day 1, day 20, 1 month, 2 months, 3 months, and 4 months, and skeletal muscle (gastrocnemius) is going to be collected with serum samples at each time points mentioned. The following measurements will be conducted to make comparisons between the wild type and transgenic groups:

1. Real-time PCR for muscle samples.
2. Western blot analysis for serum samples.
3. Immunohistochemical analysis of type II myosin heavy chain (MHC).
5. Muscle weight (gastrocnemius) (gm) after birth.

**References**

An exhaustive literature review from 37 citations was conducted and referenced in the Background statement; all sources were listed.
Status Report for Mr. Seerwan Ahmed Abdullah

PhD Candidate
Department of Molecular Biosciences and Biosystems Engineering (MBBE)
Area of Specialization: Food Science

Seerwan has been in Hawaii since October 23, 2008; and started academic study since the Spring semester of 2009. Please see the May 3 report for his course enrollment and progress. He has now completed PhD course requirements and has also undertaken research skill training with his faculty supervisor. He is fully prepared to start his PhD research. Excerpts of his dissertation research proposal are attached below.

Title

Continuous Flow Microwave and Ohmic Heating Techniques for Pasteurization of Grape Juice

Introduction

The study is an attempt to establish microwave and ohmic heating as efficient and viable pasteurization methods for grape juice. While conventional heating destroys micro-organism and the inactivation of enzyme and the subsequent extension of shelf-life of juice, on the other hand, such pasteurization method often neutralizes the dietary value as well the sensory evaluation of juice products. The experiment is design to explore alternative methods of pasteurization that will not only ensure the longevity of shelf life of juice products but also the preservation of its nutritional values and sensory quality. As such the research will illustrate that microwave and ohmic heating will effectively address the above concern. The research basically entails the experimentation of grape juice with the use of the said pasteurization methods to establish the above claims.

Background

Grapes, associates of the genus *Vitis* of the family *Vitaceae*, constitute one of the most popular fruits and are also universally cultivated in the world. According to the FAO 2002 report, grapes covered an area of about 7.5 million hectare worldwide. Grapes can be classified into two groups, namely seedless grapes, and those with seed. Besides having grape as fresh fruit, it could also be used to produce several end products. This includes raisin, grape juice, wine, jelly, and jam. Grapes are popular because it is a significant source of nutritional antioxidants, such as polyphenols, anthocyanins as well as biologically active dietary components (Orak, 2007).

Traditionally, grapes juice is often persevered through conventional heating. However, conventional heating reduces the nutritional value of grape juice. Charles-Rodriguez, et al 2007 reported, Product quality is the single most important factor to consider in the processing of product goods such as grape juice. This often includes the aroma, color, flavor and the freshness of the products. In the final analysis consumers are gravitate towards product quality. As such it is imperative to explore alternative methods of heating. According to (Tajchakavit, et al 1998), there are new technique involve in the treatment of grape juice which includes thermal and non-thermal processing. Non-thermal processing consist of ultra-filtration, high pressure process, light pulses, irradiation and electrical pulse treatment. On the other hand thermal processing involves microwave and ohmic heating. Pasteurization of grape juice products
functioned to destroy the spoilage and inactivity of enzymes such as polyphenoloxidase and peroxides in order to preserve the physiochemical and sensory tests. The latter includes taste, flavor and color.

**Merits of Continuous flow Microwave heating**

"Microwave are defined as electromagnetic waves in the assortment of infrared and radio waves, with a wavelength ranging from 1mm to 1m and functioning at a frequency of 300 MHz-3000GHz" (Thostenson & Chou,1999). Since it is an electro-thermal process, microbial devastation by microwave occurs through heat. Microwave pasteurization process is one of the alternative techniques that not only improve quality retention for liquid foods, such as fruit juices and milk, but it is also a speedy process as oppose to conventional pasteurization. The most famous properties of microwave heating are volumetric heating, unlike microwave heating, conventional heating where the heat scatters on the surface of the material. Volumetric heating means that materials can absorb microwave energy directly and within and convert it to heat (Tong, 2002).

Microwave entails two process mechanisms, specifically dipolar and ionic interactions. In specifically dipolar water is the most common polar molecule and also a major component of foods. The water molecule is a “dipole” with a positive stimulated end and a negative one. Similar to the action of magnet, these “dipoles” will familiarize themselves when they are subject to electromagnetic (Buffler, 1993). The rotation of water molecules would generate heat for cooking, whereas mechanism of microwave heating is the ions vibratory migration which is generated heat under the impact of the oscillating electric field,

The continuous flow of microwave system entails the Teflon coil in the chamber, pump, magnetrons, micro-computer and controller temperature. Juice is channeled through the Teflon coil located in the oven chamber and with preferred temperature. A number of studies have reported successful microwave pasteurization of milk, orange juice, and apple juice, involving enzyme inactivation and microbial destruction (Tajchakavit, et al, 1998., and Canumir, et al, 2002).

**Ohmic heating**

Ohmic heating is based on the channel of electrical current through a food product that has electrical resistance which eventually converts energy to heat. Immediate heating takes place depending on the current passing electricity through the substance using a variety of voltage and current combinations. Ohmic heating is used in a wide range of applications such as preheating, blanching, pasteurization, sterilization, extraction of food products (Leizerson and Shimoni, 2005a). The advantage of ohmic heating includes the maintenance of color and nutritional value of food, short processing time and higher yield as oppose to conventional heating. In conventional heating, heat is produced over a certain period of time to eventually reach the cold point of the particles and the surrounding fluid. This process often leads to the destruction of nutrients and possibly decreases the flavor of food products. Ohmic heating processes the particles and adjoining liquid simultaneously, preventing over cooking (David, 1992).

Ohmic heating is an advanced thermal process where food acts as an electrical resistor. The experimental design usually consists of electrodes that contact the food, whereby electricity is passed through the substance using a variety of voltage and current combinations. The substance is heated by the dissipation of electrical energy. Ohmic heating conducts heat throughout the entire mass of the food uniformly as oppose to conventional heating, where heat is conducted from the outside in using a hot surface. The success of ohmic heating depends on several factors. This include the rate of heat generation in the system, the electrical conductivity of the food (see: electrical conductivity), and the method by which the food flows through the system (Leizerson and Shimoni, 2005a). In the course of literature review did not find any information with regard to the use of continuous flow of microwave system and ohmic heating as a method of pasteurization of grape juice.
Rationale and Significance

Thermal processing methods are widely used for food preservation and preparation. Nevertheless, there has been an ever-increasing public concern towards the quality of these processed foods which are substandard in nutritional quality. As Lund (1975) discovered, while thermal processing reduces the activity of undesired biological materials (such as enzymes and microorganisms, e.g. bacteria, yeasts, and molds), it also destroys the essential nutrients of products such as protein coagulation, textural softening, and the formation of aroma and the loss of other components. This dilemma has generated researchers to explore alternative processing techniques to improve product quality. Continuous flow microwave heating and ohmic heating are two moderate electro-thermal treatments of grape juice in this study.

Hypothesis

This study is an attempt to explore alternative techniques of the pasteurization of grape juice which will consider the continuous flow of microwave system and ohmic heating process.

Goals and Objectives

The goal of this study is to improve a pilot scale continuous flow of microwave and ohmic heating technique for grape juice pasteurization in a small range and properties the parameters for best quality production. Specific objectives are:

1: to evaluate the effect of continuous flow microwave and ohmic heating techniques on physio-chemical properties, sensory test, and microorganisms of grape juice after pasteurization and to investigate efficient methods of pasteurization.

2: Monitor the preservation of the nutritional value of the grape juice and its shelf life for marketability.

3: Determine appropriate techniques and conditions for scaling up to commercialized plant scale.

Expected Outcome

It is anticipated that microwave and ohmic heating will be viable alternative methods of pasteurization with particular reference to grape juice. The advantages of microwave and ohmic pasteurization methods are as follows:

1. To ensure the optimum maintenance of grape nutrients in the juice and without change of the sensory tests.
2. To ensure the extension the shelf life of juice which will eventually guarantee the long term marketability of grape juice product and its economic viability of the grape juice.
3. The success of these methods will provide realistic alternatives for small food preservation businesses with modest required investments.

References

An exhaustive literature review from 22 citations was conducted and referenced in the Background statement; all sources were listed.