In vitro Generation of Superoxide by Selenofolate in MDA-MB-468 Breast Cancer Cells

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Prior studies have shown an association between generation of reactive oxygen species and some selenium compounds. Redox activity of selenium compounds is now recognized as beneficial in averting development of drug resistance, impacting the efficacy of chemotherapeutics. Several selenium compounds have been shown to be cytotoxic against cancer cells. Folate, a water-soluble vitamin that is required for DNA and RNA synthesis, and the receptors of which are upregulated in ovarian, breast, kidney and other cancer cells, was conjugated to selenium to determine if this combination produced anticancer effects. In this study, synthesized Selenofolate and the unconjugated Folate control were assayed using Lucigenin Chemiluminescence (CL) for quantifying generation of superoxide from Glutathione (GSH) oxidation. At different GSH concentrations, total chemiluminescence generated by Selenofolate was markedly increased over Folate alone which did not generate detectable superoxide. Generation of superoxide by Selenofolate was confirmed to occur intracellularly by treating triple negative breast cancer (TNBC) cells, MDA-MB-468, with Dihydroethidium (DHE), a probe that emits a red florescence indicating the presence of superoxide. Results were analyzed by fluorescence microscopy and quantitated using a fluorometric microplate reader at an excitation of 520 nm and emission of 610 nm. A significant difference in superoxide generation was observed with Selenofolate treatment over controls. These data suggest that selenium conjugation may present a strategy for the rational drug design of new anticancer pharmaceuticals.

NemaFlex: A microfluidic tool for phenotyping (neuro) muscular strength in *C. elegans*.

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Maintenance of physical fitness is essential for an individual's health and well-being. In fact, a decrease in physical fitness indicates an increased risk of hospitalization and a higher mortality risk, especially in elderly populations. While there are many aspects of physical fitness, a decline in muscle strength correlates with poor performance physically. This introduces a challenge for researchers to understand what genetic mechanisms regulate muscle strength. While these questions are difficult to probe in humans, model organisms allow these studies to be completed with ease. Specifically, *C. elegans* is a model organism that has muscle with genetic and physiological similarities to human muscle. Using *C. elegans* and a novel microfluidic platform called NemaFlex, we are able to measure the muscular strength of the nematode as an indicator of its physical fitness. The microfluidic device has been optimized for worms of various sizes. In initial tests, NemaFlex is demonstrated as an effective platform for characterizing the strength of *C. elegans* mutants with compromised muscular structures. Worm strength is a useful physical fitness metric in many types of *C. elegans* studies and has applications in obesity studies, drug screens, and more. Current obesity studies with *C. elegans* investigate the role of diet changes or drugs by looking at fat composition of the nematode or its lifespan. However, it is also useful to investigate how healthy an animal is. NemaFlex introduces a new parameter of muscular strength as an indicator of the worm's health to add to the robustness of these studies.

A Descriptive Study of the Vital Health Indicators of Four Sub-Saharan African Countries, 1960-2012

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Background: Country-level vital health statistics have the potential to initiate public health and development policies. In spite of Sub-Sahara Africa's (SSA) progress in accessibility to quality health care and improved nutrition, infant and maternal malnutrition or ill-health continue to pose enormous challenges.

Objective: To present time series analyses for selected health and wellness variables using World Bank's country level aggregated data with focus on Ghana, Togo, Burkina Faso, and the Ivory Coast.

Methods: Linear trends over time and differences among countries were assessed using simple ANOVA models, and associations were calculated using Spearman's rank correlation. The vital health indicators collected for this region can be used to analyze the health status of the vulnerable populations, epidemiological patterns, and the impact of economic growth on health outcomes; thus, we use these indicators to calculate descriptive statistics and identify trends that may inform hypotheses aimed at improving infant nutrition and maternal health.

Results: Although there has been a steep decline in infant mortality rates in all countries since 1960, those of Burkina Faso and the Ivory Coast were generally high ($p<0.001$). Life expectancy among women, while generally increasing over the period, reversed course between the mid-1980s and 2000 for all countries. All countries had several measures with less than four records across the period of interest.

Conclusions: SSA has seen a general improvement in selected health indicators, but it has been mixed and inconsistent across countries. Research efforts in this area are hindered by a lack of data reported consistently over time.

A microfluidic platform for parallelized aging and healthspan investigations in *C. elegans*

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Caenorhabditis elegans has emerged as a model organism for aging. Large-scale screens for longevity genes in *C. elegans* use liquid culture combined with drug-induced blocking of progeny, introducing physiological stress on the animals. We report a simple and high-throughput microfluidic platform addressing the limitations of current methods. The platform is capable of measuring lifespan and healthspan of wild type and mutants in parallel without a requirement for drug blocking of progeny production. This device can remove progeny efficiently while retaining adult animals in the device. In addition to standard healthspan readouts such as locomotory prowess and pharyngeal pumping, the device allows recording of novel measures such as muscle strength and agility.

We test the multifunctional capabilities of the device by conducting a pilot screen that includes wild-type, *daf-2*, *daf-16*, *age-1* and *eat-2* animals. We find that the lifespan curves of the wild-type and genetic mutants are consistent with the literature reports. We also show that in a targeted RNAi screen, the lifespan curves obtained were consistent with those of the genetic mutants. Analysis of muscle strength, agility, and locomotory prowess as a function of lifespan in the long-lived mutants reveals new insights into sarcopenia. Moreover, easy control over the feeding system made the device suitable for nutritional studies. This has been shown by inducing dietary restriction (DR) on wild-type worms. The efficacy of DR has been demonstrated through the extension of worm lifespan and development. We anticipate that our device will enable highly parallelized cross-sectional and longitudinal aging experiments.

Metabolic Changes Underlying Interactions Between Obesity and Non-Alcoholic Fatty Liver Disease in South Asian Adults

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Non-alcoholic fatty liver disease (NAFLD) is often referred to as the hepatic manifestation of the metabolic syndrome. However, not all human subjects with obesity develop metabolic complications. In addition, it is unclear why South Asian adults develop insulin resistance and diabetes even at lower levels of adiposity and body mass index compared to their Western counterparts. To address this question, we used a convenient sample of Sri Lankan adults (n = 30) who underwent routine abdominal surgery at the Teaching Hospital of Peradeniya. Anthropometric data, adipose tissue specimens and fasted serum samples were collected for analyses of glucose, lipid and inflammatory markers. Histological sections of adipose tissue were obtained from omental fat (OF) and anterior abdominal wall fat (AF), and adipocyte cross-sectional area was measured using the ImageJ software. Further, the presence of hepatic steatosis was assessed using ultrasound scanning by a radiologist. Participants with higher grade of fatty liver had a significantly higher body mass index (BMI), fasting blood glucose and aspartate aminotransferase levels. Additionally, participants with higher grade of fatty liver also had significantly higher serum levels of the pro-inflammatory adipokine, resistin. Interestingly, there were no significant differences in serum adiponectin levels between the groups. Individuals with fatty liver also had a higher mean adipocyte area in both OF and AF, indicating a higher degree of adipocyte hypertrophy associated with fatty liver. These findings indicate distinct metabolic differences between South Asian adults with varying degrees of fatty liver, and may help in establishing effective treatment strategies for obesity-associated NAFLD.

Transcriptomic and MicroRNA Analyses Identify Gene Networks Regulated by Eicosapentaenoic Acid in Brown Adipose Tissue from Diet-Induced Obese Mice

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Brown adipose tissue (BAT) dissipates chemical energy as heat and protects against obesity by increasing energy expenditure. In this study, we used RNA sequencing (RNA-Seq) and microRNA (miRNA) profiling, as powerful methods to identify novel regulators of BAT transcriptome. These techniques were applied on BAT from C57BL/6J mice fed either a high fat diet (HF, 45% kcal fat) or HF diet supplemented with EPA (HF-EPA containing 36g EPA/kg diet) for 11 weeks. RNA sequencing was performed using Illumina Hi-Seq and 831 genes were identified that were differentially expressed (95% confidence and $p<0.05$) in BAT from HF compared to HF-EPA fed mice. HF-EPA had significantly higher expression of genes in fatty acid oxidation and thermogenesis such as peroxisome proliferator-activated receptor-α (Ppar-α), retinoid X receptor (Rxr), phosphatase and tensin homolog (Pten) and reduced expression of inflammatory genes such as nuclear factor kappa-light-chain-enhancer of activated B cells (Nf-κb), and Tumor necrosis factor receptor 1 (Tnfr1). Moreover, miRNA profiling identified nine upregulated miRNAs and six downregulated miRNAs by EPA respectively. MiRNAs identified including miR-455-3 and miR-150-5p are key regulators of BAT thermogenic function. In summary, combining RNA-Seq and miRNA profiling help to identify novel biomarkers mediating nutritional regulation of thermogenesis.

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Could FTIR Imaging be used to Detect Lipid Accumulation in Adipose Tissues?

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Obesity is a complex disease that has become highly prevalent and associated with several chronic metabolic diseases. We are interested in dietary interventions that may alleviate obesity and its associated lipid disorders. Intracellular lipid accumulation is commonly studied by histology, using chemically fixed paraffin embedded tissues. However, the chemical fixatives and the common deparaffinizing agents tend to remove lipids, making the investigation related to lipid distribution in biological sections inaccurate and practically impossible. Therefore, we propose to use cryosectioning method as an alternative in histological studies, because chemicals are not extensively utilized. Developing a reliable cryosectioning protocol is of utmost importance to obtain accurate distribution of lipids within the tissues. Fourier Transformed Infrared microspectroscopy Imaging (FTIRI) is a powerful, nondestructive, and staining-free technique, which is capable of exploring the structural and compositional variations in biological tissues. In this project we (1) optimized the cryosectioning process of adipose tissues harvested from mice fed low-fat and high-fat diets and (2) employed FTIRI to study the variation in macromolecular composition at the cellular level due to a high-fat diet. Our results show that the intensities of the infrared vibrations assigned to lipids increase with high-fat diet. This demonstrates that cryosectioning followed by FTIRI could be used to study dietary induced lipid accumulation within tissues. Liver and adipose tissues harvested from mice fed high-fat diet supplemented with omega-3 fatty acids will be also studied, which will help us to investigate the effect of dietary changes on tissue composition.

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Vitamin D reverses glucose metabolism by regulating mammalian target of rapamycin (mTOR) in breast cancer cells

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Background and Objective: Breast cancer accounts for the second most common cancer related deaths in the USA. Cancer cells rely on aerobic glycolysis for energy production, growth and survival. Vitamin D is not only important for bone mineralization but also improves insulin resistance, prevents inflammation, and macrophage foam cell formation. Additionally, studies showed lower levels of serum vitamin D in breast cancer patients. However, it remains obscure whether vitamin D regulates glycolysis and inhibits breast cancer cell proliferation.

Methods: MCF-7 (estrogen receptor (ER) positive) cells were grown in DMEM high glucose medium and treated with and without (control) vitamin D (calcitriol) for 24 hours. Cells were harvested and processed for Western blotting, RNA extraction, quantitative-PCR and cell viability assay.

Results: Vitamin D treatment decreased the viability of the cancer cells after 24 hours of treatment. In addition, expression of key glycolytic proteins including glucose transporter 1 (GLUT1), hexokinase II (HKII) and lactate dehydrogenase A (LDHA) were reduced. Furthermore, vitamin D induced apoptosis by upregulating the cleaved caspase 9 protein expression. Interestingly, expression of mammalian target of rapamycin (mTOR) that regulates glycolysis and cell survival in cancer cells were suppressed upon vitamin D treatment which was associated with the 5' adenosine monophosphate-activated protein kinase (AMPK) activation (regulates energy metabolism).

Significance: These results suggested that vitamin D is an important regulator of glucose metabolism and cell proliferation in breast cancer and may very likely have therapeutic potential.

Conclusion: Vitamin D regulates glycolysis in MCF-7 cells by targeting mTOR.

Adipose Depot-Specific Differences in Transcriptome and MicroRNA Expression in High Fat Diet Induced Obese Mice

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Obesity is associated with expansion of white adipose tissue (WAT), and contributes to diabetes and cardiovascular disease. Two main types of adipose tissue are WAT (subcutaneous, SAT and visceral adipose tissue, VAT) and brown adipose tissue (BAT), which exhibit anatomical, physiological and metabolic differences. Understanding the differences in transcriptomes and microRNA (miRNA) signatures of adipose tissue depots would help to gain mechanistic insight regarding their contribution to metabolic disorders in obesity. For this purpose, we performed RNA-sequencing of SAT, VAT and BAT depots from high fat diet (45% kcal from fat)-induced obese mice. Functional analyses revealed inflammatory pathways (such as cytokine and integrin signaling) increased in VAT compared to BAT. In contrast, pathways involved in fatty acid oxidation, apoptosis and energy expenditure (peroxisome proliferator activated receptor (Ppar)/ retinoid X receptor (Rxr-α)) were higher in BAT than in VAT. Several genes including X-box binding protein-1 (Xbp1) of unfolded protein response (UPR) pathway, which are activated following endoplasmic reticulum (ER) stress, were upregulated in VAT than in BAT. We observed miRNA-mRNA pairs involved in UPR such as Xbp1-miR-30c-2-3p and miR-455-calreticulin. Furthermore, miRNAs miR-221-3p and miR-222-3p, related to higher inflammation and ER stress were activated in VAT compared to BAT. In summary, high fat diet differentially regulate specific miRNAs and gene expression in different adipose tissue depots with significant induction of ER stress, UPR and inflammation in VAT.

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Loss of C/EBPβ attenuates atherogenic lipid mediated induction of ER stress and apoptosis but increases autophagy activity

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Background: Atherosclerosis is a leading cause of death in western societies. Accumulating evidence suggests that macrophages take up excessive atherogenic lipids and form foam cells. These foam cells can stimulate the production of inflammatory cytokines, induce endoplasmic reticulum stress (ERS), and apoptosis and can lead to atherosclerosis development. Autophagy is a catabolic pathway and has been shown to regulate cholesterol homeostasis in atherosclerosis. CCAAT/enhancer binding protein β (C/EBPβ) is a transcription factor and an important regulator of inflammation and ERS. However, whether C/EBPβ regulates inflammation, ERS and apoptosis in foam cells via autophagy is not known.

Objective: To determine whether C/EBPβ regulates inflammation, apoptosis, and ERS in macrophage foam cells via autophagy machinery.

Methods: RAW 264.7 macrophage cells were transduced with control-siRNA (50 nM) and C/EBPβ-siRNA (50 nM) for 24 h followed by treatment with nLDL or oxLDL (20 µg/ml) for an additional 24 h. Expression of genes and proteins were analyzed by qRT-PCR and western blot respectively. Oil Red O staining was employed to detect lipid accumulation.

Results: Knocking down of C/EBPβ in macrophage cells prevented atherogenic lipid mediated foam cell formation and decreased the expression of genes implicated in inflammation, ERS and apoptosis. Interestingly, C/EBPβ knock down increased the expression of autophagy marker protein (LC3II).

Significance: The findings may provide a more specific target to prevent foam cell formation and atherosclerosis.

Conclusion: The present results suggest that C/EBPβ regulates inflammation, ERS and apoptosis in macrophage foam cells in part by increasing autophagy.

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Radiation Dose Using a New Geometry of Women's Breast for Mammogram X-Ray Exposure

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Exposure to ionization radiation has been increasing gradually in our society due to the increase in the use of medical equipment's. Mammograms have been more frequently used to detect breast cancer at an early stage. The mammogram procedure consists of taking X-ray pictures of the breast from different angles. The exposure from the X-ray itself is not harmful and there is no direct evidence at this exposure level that cancer will develop. However, studies have shown that the cancer risk is greater for women age 40 years or older with no family history of cancer after using a mammogram on an annual basis. In this study, a code was developed using MCNP6 (Monte Carlo N-Particle transport code) to estimate the absorbed dose in different region of the breast. Compressed breast thicknesses of 4 cm, 6 cm, 8 cm and 10 cm were considered during the dose calculation with varying composition of adipose and glandular tissue. A proper model of the breast was developed using semi-elliptical macro-body command in MCNP6 to accurately simulate a breast phantom during the mammogram test. The aim of this work was to develop a MCNP geometry of the breast, which will resemble an actual compressed breast as accurately as possible to derive valid simulation results of a mammogram test. In addition to this, a secondary aim was to observe how change of different parameters such as kVp (Kilo Volt peak) and mAs (Milliampere Second) variation, change of compressed breast thickness, radius and tissue composition affects the energy deposition in breast.

Self-reported barriers to adapting contextualized nutrition education messages targeted at preventing iron deficiency among mothers and their young children in Ghana

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Objective: Nutrition education has the potential to improve nutritional status if focused on food sources for prevention of iron deficiency (ID). This study intended to assess the barriers of adherence to nutrition education intervention messages on iron deficiency among mothers of young children in Northern Ghana.

Methods: A baseline survey on 161 randomly selected mothers with children <5 years from two randomly selected districts in Northern Ghana, conducted with a multi-method approach in April 2012, identified gaps knowledge gaps regarding ID which was subsequently addressed in key messages during a 5-day (90minutes/day) nutrition education intervention. Ten key messages were delivered through practical, pictorial and interactive session with 73 mothers in the randomly selected intervention district in July, 2013. Mothers were visited twice (14 days apart) in their household to refresh and integrate the messages into each individual household situation. Three months post-intervention, a questionnaire designed by the researcher to assess recall of messages, adherence and challenges of implementation, was administered in structured interviews and responses were categorize into themes by content analysis.

Results: Of the 71mothers, most were married, had no formal education (98.6%), and lived in households with low median monthly income of 40 cedis ($13.3), mainly from farm produce. Of those who responded, 6 (8.8%) could not recall any message, 58 (85.3%) could recall 1-5 messages, 4 (5.9%) could recall 6-9 messages and none of them could recall all ten messages. The highest recall was on messages regarding the *prevention of malaria, diarrhoea and worm infestation which can increase risk for ID (69.9%).* This may be due to high malaria infestation in the community; thus the mothers could relate to these messages. About a third (27.4%) of the mothers reported that they now fortified the children's food with fish and 5.5% indicated having increased portions of soup to children, compared to baseline. The highest reported (40.8%) implemented messages were on regular handwashing with soap before cooking. Financial constraints was reported as the reason for not implementing messages on eating iron sources (meat/fish) and improving food choices. Messages which required behavior change, for example regarding pica practice, where not implemented, nor relayed to others in the household, due to forgetfulness, shyness, lack of support, and fear of being seen as a "know it all". Some reported that infants did not like, or refused to eat foods fortified with fish and groundnuts.

Conclusion: Messages for nutrition education, even when contextualised, still pose barriers to recall and adherence. In this rural setting in Northern Ghana, mothers' social environment seemed to play an important role, suggesting that behavior change models targeted at building the confidence of mothers to act as change agents in their households and community, may be more successful.

Keywords: Barriers, contextualized nutrition education, Iron deficiency anemia, Ghana

Xenotransplanted Sertoli Cells Inhibit both Alternative and Classical Pathways of Complement Mediated Cell Lysis

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Type I diabetes mellitus (TIDM) is an autoimmune disease where the body's own immune system kills the insulin-secreting islet cells of the pancreas. It can lead to chronic complications such as cardiovascular disease, kidney failure, lower-limb amputations, and blindness (Craig et al, 2014). Current treatment for TIDM is insulin therapy (insulin shots), however, it is costly and does not sufficiently control blood glucose levels; thus leading to complications associated with this disease. Diabetic women have a 3.5 and 5 times higher risk of developing cardiovascular complications and heart disease, respectively, compared to diabetic men (Franconi et al., 2012)(Ren and Ceylan-Isik, 2004). However, diabetic men are more likely to undergo lower extremity amputations, develop peripheral vascular disease, and peripheral neuropathy (Peek, M.E. 2011). This suggests a gender disparity is present needing further investigation and early treatment to control glucose fluctuations is imperative in successful long term treatment.

One treatment option involves transplanting functional human islet cells into diabetic patients. While promising, this procedure cannot be used to treat most patients with diabetes. Difficulties are associated with obtaining sufficient human donor tissue and life-term dependency on immune suppressive drugs to prevent the rejection of the transplanted islets. Xenotransplantation, pig tissue into humans, has the potential to solve the problem of donor tissue shortage. Yet, xenotransplantation is currently limited by potent immune responses against xenogeneic tissue, which is not clearly understood. Interestingly, we have demonstrated that neonatal pig Sertoli cells (NPSC) survive xenotransplantation up to 90 days without the use of any immune suppressive therapy. This suggests that understanding the survival mechanism of NPSC will help identify the key factors that are required to negate the immune response generated against neonatal pig islets (NPI).

Eleven million NPSC or non-immune privileged NPI were transplanted into rats. Grafts were collected at days 1-20 post-transplantation. Cell survival analysis revealed NPI were completely rejected within 9 days, while NPSC survived throughout the study. Binding of natural and induced antibodies and activation of complement cascade are part of the xenograft rejection mechanism. To test this, NPSC or NPI grafts and serum from the transplanted animals were collected, and analyzed for antibody production and deposition, and complement factor (C3 and MAC) deposition. There was no significant increase in IgM production against NPSC or NPI compared to pre-transplanted values. IgM deposition was detected from day 6 onwards in both NPSC and NPI grafts. A significant increase in IgG production was observed at days 13 and 20, in case of NPSC, and day 20, in case of NPI. IgG deposition was first detected at day 9 in NPSC grafts and day 13 in NPI grafts. C3 deposition was identified at days 1 and 3 in NPI grafts and only at day 1 in NPSC grafts. MAC deposition was also detected at early time points, days 1-4, in NPI grafts while no MAC deposition was identified in NPSC grafts. Collectively, these data suggest that activation of the alternative pathway of complement cascade releases anaphylotoxins thereby recruiting other immune cells to aid in NPI graft destruction. NPSC actively inhibit both the alternative and classical pathways of complement mediated cell lysis.

The physical stability comparison of two types of resveratrol nanocarriers

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Objective Resveratrol (R) has anti-obesity bioactivities, but its low level of aqueous solubility and stability limits its application. We successfully synthesized biocompatible and biodegradable R encapsulated lipid nanoparticles (R-nano) and R encapsulated liposomes (R-lipo). The objectives of this study are to measure the characteristics of R-nano and R-lipo and to compare their physical and chemical stability at three different temperatures.

Method The particle size and polydispersity index (PI) of R-nano and R-lipo were measured using a Brookhaven BI-MAS particle size analyzer, and their zeta potential were measured using a Brookhaven ZetaPALS analyzer. The R-nano and R-lipo were stored in dark at 4°C, 22°C and 37°C for 7 days, and their physical and chemical stability was measured every 24 hours. The freshly made R-nano and R-lipo were aliquot into black tubes and stored at the above three temperatures. The mean particle size, PI and zeta potential were measured every 2 hours for the first 10 hours, and every 24 hours for 7 days. The chemical stability of R-nano, R-lipo and native R were measured by a high-performance liquid chromatography system.

Results The mean particle size of freshly made R-nano and R-lipo were around 140 nm and 110 nm, respectively. The PI values of both R-nano and R-lipo were less than 0.3. Moreover, the zeta potential of R-nano and R-lipo were around −19 and −28mV. As compared to native R, R-nano and R-lipo dramatically increased the aqueous solubility of R by more than 30 times. Nanoencapsulation also significantly enhanced the chemical stability of R. The diameter and zeta potential of R-lipo did not changed significantly at all temperatures (4°C, 22°C and 37°C) for 7 days, and the PI of R-lipo remained under 0.3. R-nano had lower physical stability than R-lipo at 22°C and 37°C. After incubating at 22°C for 24 hours and at 37°C for 8 hours, the particle size was dramatically increased, and their PI and zeta potential values were also increased. The particle size, PI and zeta potential of R-nano were stable at 4°C for 24 hours.

Conclusions Both R-lipo and R-nano increased the aqueous solubility and stability of R. R-lipo had higher physical stability than R-nano. We are investigating the anti-obesity bioactivities of nanoencapsulated R.

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The characteristics and stability Of T0901317 nanovesicles

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Introduction T0901317(T090) is a potent synthetic agonist for the liver X receptors, which activation could reduce the atherosclerotic lesion size. However, T090 can also induce liver steatosis, which hinder its clinical application. Intimal macrophages are determinant cells for atherosclerotic lesion development. We have successfully made T090 nanovesicles (Tnano) and ligand-incorporated Tnano (LTnano) for targeting intimal macrophages. The objective of this project is to measure the characteristics and stability of Tnano and LTnano.

Methods The size and polydispersity index (PI) of Tnano and LTnano were measured using a Brookhaven BI-MAS particle size analyzer, and their zeta potential was measured using a Brookhaven ZetaPALS analyzer. The encapsulation efficiency of T090 was measured using a filtration method and a high performance liquid chromatography system (HPLC). Tnano, LTnano and free T090 were incubated at 4°C, 22°C, 37°C. Their physical and chemical stability was measured every 2 hours for 24 hours. A dialysis method was used to investigate the release pattern of the free and nanoencapsulated T090. Tnano, LTnano, free T090 containing 0.4 mg of T090 were put in three different dialysis bags, which were soaked in a sink solution made of 1X PBS containing 10% methanol. Sink solution was collected and changed every 2 hours for 24 hours. The T090 concentrations in the sink solution were measured using a HPLC method.

Results The mean particle size of Tnano and LTnano was less than 100 nm, the PI of Tnano and LTnano was less than 0.3, the zeta potential of Tnano and LTnano was around -30. The encapsulation efficiency of T090 was more than 90%. Nanoencapsulation increased the aqueous solubility of T090 by more than 1,000 times. Nanoencapsulated T090 is more stable than free T090. Tnano and LTnano demonstrated a sustained release manner.

Conclusion Nanoencapsualtion increased the aqueous solubility, biostability and releasing sustainability of T090. The anti-atherosclerotic activities are under investigation.

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Tocotrienol dose-dependently improves adiposity and inflammation and increased markers of lipid oxidation in high fat fed mice

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Obesity is a disease that affects one in three American adults. Inflammation has been established as a major underlying basis for obesity and related chronic disorders. Several plant bioactive compounds with anti-inflammatory properties have been used to reduce obesity and associated metabolic complications. Hence we are investigating the effect of tocotrienol (T3), a member of the vitamin E family as an anti-obesity agent. We hypothesize that T3 will reduce obesity and insulin resistance through their anti-inflammatory and anti-oxidant properties. To test our hypothesis, we used C57BL/6J male mice that were fed a high fat diet without (HF) or with supplementation of increasing levels of tocotrienol (HF+T3) up to 1,600mg/kg for 14 weeks. Glucose and insulin tolerance tests were administered two weeks prior to the end of the treatments. Serum and tissues including adipose tissue were collected at the end of the study. Our results show significant improvements in glucose clearance in the T3-supplemented groups compared to the HF group. Fat pad weights were reduced dose-dependently by T3. These changes were also associated with smaller fat cell size and reduced crown like structures associated with macrophages in adipose tissue histology sections from HF+T3 compared to HF-fed mice. We also performed mechanistic analyses that revealed reduced mRNA and protein expression of pro-inflammatory adipokines including resistin, leptin, and MCP-1 and increased expression of anti-inflammatory adipokines such as adiponectin and IL-10 in HF+T3 compared to HF-fed mice. Moreover, T3 dose-dependently increased fatty acid oxidizing genes. In summary dietary tocotrienols exert promising anti-obesity and anti-inflammatory effects that may be mediated in part by adipose tissue.

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