Medical Student Research Day

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ABSTRACTS
Comprehensive and tailored sex education workshop increases the knowledge of sexual health practices among Native American youth participants.

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Although teen birth rates and teen rates of sexually-transmitted diseases (STDs) in the United States have declined, they remain high among racial-ethnic minority youth in the southern states. Sex-education programs and workshops for youth have been effective in reducing STDs, increasing contraceptive use, and decreasing teen pregnancy rates. Hence, this project conducted a sex-education workshop with Native American youth of Halifax and Warren County in North Carolina. The standard sex-education workshop was used, and focused on postponing sexual activity, decreasing the frequency of sexual activity, decreasing the number of sexual partners and of unprotected sex, and increasing use of condoms and contraceptives. The sample consisted of 26 teens ages 13 to 18 years, boys and girls. They completed a survey of their sexual behavior and attitudes before the sex-education workshop and then again after the workshop. Results revealed that before the workshop, 65% of teens thought that having a sex-education program was important whereas after the workshop, 100% thought that sex-education for teens was important. In addition, the percentage who believed that their county has a high rate of teen pregnancies increased from 11% before to 60% after the workshop. These and other findings suggest that the workshop increased knowledge among the participants.
Colorectal cancer cell adhesion and metastatic potential significantly decreased after exposure to ceramide
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Background: Colorectal cancer (CRC) can become highly metastatic, responsible for more than 1 million cases of newly diagnosed cancer every year and is the fourth most common cancer cause of death in the world. Ceramide, the neutral lipid backbone of cellular sphingolipids, has been shown to be a powerful cancer suppressor, especially as regards CRC cells; however, the impact of ceramide on metastatic potential is unknown.

Hypothesis: Because ceramide is an efficient tumor suppressor, we hypothesized that its effectiveness may, in part, be related to its effects on cellular adhesion and migration. As little is known about the structural requirements of ceramide, we also hypothesized that characteristics such as physical presentation and saturation are important factors for its ability to blunt metastasis.

Methods: HT-29 human CRC cells were treated for 24 hr. with 5µM or 10 µM of D- or L-C6 ceramide in a DMSO vehicle, D-C6 ceramide in a nanoliposome, DMSO alone, empty nanoliposomes alone (Ghost), dihydroceramide, and monoolein. The treated cells were then used in 1-hr adhesion assays using fibronectin-coated 96 well plates, and 24-hr migration assays using 8µm-pore transwell cups. Adhesion assays were quantified using calcein AM and a microplate reader measuring the fluorescence. Migration assays were quantified using crystal violet, 1% SDS, and 50% ethanol, measuring colorimetrically.

Results: Our investigation revealed that: i) C6 ceramide is a powerful inhibitor of adhesion and migration of HT-29 cells; ii) when delivered in a nanoliposome, ceramide was shown to be as efficient, if not more, than when delivered in DMSO; iii) in comparison to the natural D- isomer of ceramide, the unnatural L- isomer displayed no effect; iv) the unsaturated form of ceramide, dihydroceramide, had greatly reduced efficacy; and v) lacking the presence of the six carbon chain, monoolein increased the cells’ ability to adhere and migrate.

Conclusion: These results suggest that the D-isomer of C6 ceramide significantly decreases both adhesion and migration in HT-29 CRC cells. When delivered using a nanoliposome vehicle, the decrease was even greater. Furthermore, physical characteristics such as saturation and optical presentation are important for this effect. Finally, the results also suggest that this effect is due to the specific structure of C6 ceramide, and likely not due to nonspecific lipid interactions.

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Effect of Treatment with p1158/59 Matricryptin on Early Autologous Vein Remodeling in Arterial Bypass Grafting

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Background: Arterial bypass graft surgery is currently the most common procedure used to restore blood flow in patients with atherosclerosis related diseases. Despite their prevalence, 60-70% of vein grafts will fail within ten years and need to be replaced. Increased collagen can be a source of active cleavage fragments that modulate growth responses. Treatment with p1158/59 matricryptin, one such cleavage peptide, has been shown to stimulate angiogenesis and improve ventricular recovery in a mouse myocardial infarction model.

Objective: Given the role of collagen and extracellular matrix remodeling that occurs in veins after arterial grafting, these experiments were developed to test the hypothesis that treating veins with p1158/59 prior to grafting would modulate early growth responses (within 10 days) consistent with better graft patency.

Methods: A canine model (n = 12; 6 male, 6 female, 25-35 kg) of carotid artery bypass was used. Ultrasound of the carotid circulatory anatomy prior to, 5 and 10 days after bypass grafting was performed. For bypass procedures, animals were anesthetized, intubated and ventilated with 1-3% isoflurane, oxygen and room air. A lateral hind limb incision was made, and approximately 12-15 cm (in situ) of saphenous vein was harvested. The vein was divided with one half serving as control, and the other half incubated (1.5 hrs) in a solution containing p1158/59. Treated and untreated veins were anastomosed end-to-side into the common carotid artery. Once graft patency was established, the native artery was divided, all surgical wounds were repaired, and the animals were recovered. 10 days later, animals were anesthetized as before, the implanted vein grafts were removed, and sections of the graft were allocated for gene expression (PCR array), histology, and primary cell culture to assess characteristics of smooth muscle cell migration and adhesion.

Results: Primary cell cultures were successfully grown to confluence and plated for migration studies, which are ongoing. Preliminary western blotting of the cells has verified that they are smooth muscle cells. Gene expression and histology results are pending.

Conclusion: Visual inspection of the grafts at the time of harvest suggests that there was a treatment effect of the matricryptin peptide, but more definitive conclusions are pending the outcome of ongoing data analysis.

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**Relationship Between Diabetes-Related Distress and Glycemic Control in Patients with Type 2 Diabetes**

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**Objective:** Previous studies have shown that high levels of diabetes-related distress are associated with elevated HbA1c in patients with type 2 diabetes, however, the potential explanatory factors associated with this relationship are not well known. The purpose of this study is to analyze the relationship between diabetes-related distress and medication adherence, nutrition, exercise, and self-efficacy in patients with a wide range of elevated HbA1c values.

**Methods:** In this cross-sectional study, the following data was collected at baseline for the COMRADE study: diabetes distress levels using the 17-item Diabetes Distress Scale (DDS-17), HbA1c using a DCA Vantage Analyzer, medication adherence using the Morisky Medication Adherence Scale (MMAS), nutrition using the Rapid Eating and Activity Assessment for Patients (REAP), exercise using the Duke Activity Status Index (DASI), and self-efficacy using the Diabetes Empowerment Scale-Short Form (DES). Based on mean DDS-17 scores, patients were categorized into two groups: low distress (DDS-17 <3.0) and high distress (DDS-17 ≥3.0).

**Results:** Ninety-two (n=92) patients were enrolled in this study with the following characteristics: mean HbA1c = 9.6% ± 2.0%, mean BMI = 37.1 ± 9.9, mean age 53 ± 9.8 years, 72% African-American, and 73% female. Seventeen percent of patients reported elevated diabetes-related distress, with increased prevalence among older patients currently taking insulin and with a longer T2DM duration. Compared with patients with low levels of diabetes-related distress, those with elevated levels of diabetes-related distress had higher mean HbA1c (p<0.05) and lower mean self-efficacy (p<0.05), medication adherence, physical activity scores (p<0.05), and adherence to a nutritional diet. Using Pearson’s correlation, elevated DDS-17 scores were significantly correlated with higher HbA1c (r=0.274; p<0.01), lower DES (r=-0.423; p<0.01), lower MMAS (r=-0.380; p<0.01), and lower metabolic equivalents (r=-0.209; p<0.05).

**Conclusion:** Among patients with type 2 diabetes in a rural setting, elevated diabetes-related distress and HbA1c were associated with lower medication adherence, poor diet, decreased physical activity, and lower self-efficacy. These findings suggest that distress may be associated with inadequate engagement in appropriate self-care behaviors. Findings in this study can be used to help implement a collaborative care intervention to improve diabetes-related distress and glycemic control.
Influence of Maternal Activity on Child Oral Health and Tooth Development

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Background: The physical health of a pregnant woman is associated with positive outcomes for her child. Studies have shown healthier hearts and less obesity in children of women who were active prenatally. The link between pregnant women exercising and their children’s oral health and tooth development has not been researched.

Objective: We sought to evaluate the relationship between physical activity during pregnancy and childhood oral health and development. We hypothesized that women who exercised while pregnant would have children with lower dental disease progression, dental caries risk, and increased tooth maturation.

Methods: Women who had children six years of age or younger seen as a patient at East Carolina University School of Dental Medicine Pediatrics Clinic were asked to participate in the study. Participants were asked 1.) to complete a questionnaire about their prenatal activity and 2.) for consent to their child's dental records.

Results: Statistical analyses included t-tests and ANOVAs. No significant differences were found between groups in Age or BMI of mothers or children. There are no differences between groups for decreased dental disease progression or lowered dental caries risk in children. A trend toward increased tooth maturity as a function of child’s age was found related to prenatal activity.

Conclusions: The data suggests that variation in behaviors postpartum limit any association between prenatal exercise and lowered offspring dental disease progression and caries risk. A physiological link is seen between child dental maturity and maternal activity possibly due to the child’s increased nervous system development. Clinicians should focus on the postpartum behaviors to improve childhood oral health.
Impact of exercise on vascular collateralization after hind limb ischemia in rats bred for high and low aerobic capacity

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**Background:** Peripheral arterial disease (PAD) impacts one in 20 Americans over the age of 50. Active exercise has been reported as having prophylactic benefit in PAD, and is also considered a central part of recovery following interventions for PAD. Less well understood is the relationship between latent genetic capacity for exercise and active exercise in PAD outcomes. Vascular collateralization is key to the survival of the impacted tissues; thus, understanding how to increase collateralization and the role of both active exercise and genetic capacity will help improve the prognosis and course of treatment for patients with PAD.

**Objective and Hypothesis:** To determine whether exercise prior to or exercise after femoral artery ligation induces a greater level of compensatory vascular collateralization and the role, if any, of intrinsic exercise genetic background in the collateralization response. I hypothesize that exercise post-ligation group will show increased expression of angiogenic factors and a higher capillary density suggesting a more successful compensatory vascular collateralization, and that this response will be greater in the high aerobic capacity group.

**Methods:** 34th generation female rats, phenotypically selected for either high or low aerobic endurance running capacity (HCR and LCR), were assigned to four groups: control, exercise pre-ligation, exercise post-ligation, and Bendavia injection. Both exercise groups were acclimated to the treadmill for five days at 10 m/min. The exercise protocol was 14 m/min for 5 minutes (0 grade), increasing by 5 min/day for 10 days, either before ligation, or beginning post-op day 3 following ligation. Peripheral ischemia was achieved using one simple suture ligation of the proximal femoral artery. Humane termination and tissue collection occurred on post-op day 14. A comparison between the gastrocnemius from the ischemic leg and the non-ischemic leg will be completed through an angiogenesis PCR array and Rosenblatt stained histology specimens. The histology specimens will be used to determine capillary density.

**Results:** PCR and histology results are pending. Qualitatively, there was a visible difference in the sizes of the ischemic and non-ischemic gastrocnemius muscles at the time of harvest. Additionally, there was a distinct difference between the fiber makeup in the ischemic and non-ischemic muscles with the ischemic side showing significantly less red-fiber muscle than the non-ischemic side.

**Conclusion:** Pending further analysis, the ligation method successfully created an ischemic environment that significantly impacted the muscle, but the specific impact of active exercise timing, or the contribution of latent exercise capacity remains to be determined.

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Patient Attitudes, Perceptions and Treatment Preferences for End-of-Life Care

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Background: North Carolina’s Medical Orders for Scope of Treatment (MOST) form, much similar to Oregon’s Physician Orders for Life Sustaining Treatment (POLST) form, has been associated with more accurate documentation of patient treatment preferences in regards to End-of-Life (EOL) scenarios. The MOST form has also been associated with administration of EOL care that is more consistent with previously stated patient treatment preferences and goals.

Objective: The purpose of this study was to determine patient perceptions and knowledge levels in regards to advance directives and EOL care. This study also aimed to increase patient awareness, and utilization, if appropriate, of the NC MOST form.

Methods: Patients 65 and older were recruited at an academic outpatient Family Medicine clinic in North Carolina to complete a 23-question survey designed to elicit patient attitudes and knowledge levels towards advance directives and EOL care. Patients were also briefly educated by a medical student about the purpose of the NC MOST form, and given further educational material regarding advance directives upon request.

Results: While 94% and 77% of patients were familiar with Health Care Power of Attorney (HCPOA) and Do Not Resuscitate forms, respectively, only 7% of participants reported familiarity with the MOST form. Upon completing the survey, 52% of participants responded affirmatively when asked about interest level in discussing Advance Directives or completing a MOST form. Out of 200 survey participants, 95 participants requested further educational materials in the form of a sample MOST form or an educational packet addressing HCPOA and living will completion.
Demographic and historical variables predict incorrect use of opioids in chronic pain patients who present to the Emergency Department

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Background: Each month 4.5 million Americans engage in non-medical use of prescribed pain medication. At particular risk of inappropriate opioid use are the 100 million US adults in chronic pain, who are 5 times more likely to present to the ED than other patients.

Objective: The objective of this study was to identify characteristics of chronic pain patients presenting to the ED who are at risk of incorrectly using prescribed opioids. We hypothesize that there are specific biopsychosocial attributes that will effectively predict an individual’s likelihood for incorrect use of opioids.

Methods: Forty-one ED patients with a chief complaint of chronic pain were enrolled. Researchers administered 3 questionnaires, obtained a voluntary urinalysis, and reviewed medical and ED encounter information. Participants were classified using each of these three models of incorrect opioid use: (1) abusing or non-abusing (DAST-10), (2) opioid use disorder or non-opioid use disorder (DSM-V), (3) aberrant behavior or non-aberrant behavior (ADTB). Each model was analyzed by bivariate statistics and a multivariate logistics regression. All procedures were reviewed and approved by the UMCIRB.

Results: Our sample was 39% female, 54% African American, with an average age of 42.4 ± 13.9 years. The significant predictor for abusing opioids was being insured by Medicare or Medicaid (OR 5.8 95% CI 1.0 – 31). The only significant predictor for both opioid use disorder and aberrant drug taking behavior was if the participant engaged in non-authorized dose increase (OR 7.7 95% CI 1.1 – 56; OR 16.3 95% CI 2.1 – 127, respectively). No variable studied was predictive of incorrect opioid use across all 3 models tested.

Conclusions: We demonstrated a discrepancy between predictor variables and standard definitions of incorrect opioid use. Our findings suggest that ED physicians may need to consider the patient’s drug use history as well as insurance status before prescribing opioids in order to ameliorate the opioid crisis.
Development of bovine platelet-rich plasma as a scaffold for cell-based cartilage repair models of osteoarthritis

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Introduction: Osteoarthritis (OA) is a debilitating progressive joint disease for which there is no long-term treatment, bar invasive arthroplasty procedures. One of the major drawbacks in treatment of OA is the lack of blood supply and the auto-catabolic nature of injured cartilage. This point highlights the need for a viable cell-based therapy treatment. However, further development of a scaffold to hold the cells in the cartilage defect is needed. Additionally, it is immunologically advantageous to develop such a scaffold from an autologous tissue source. Platelet rich plasma (PRP) has been used in various applications and is known for its richness in numerous growth factors.

Methods: PRP was prepared from acid citrate dextrose (ACD) anticoagulated whole bovine blood using the double spin method. The top 2/3 of plasma was removed and deemed platelet-poor-plasma. The remaining bottom 1/3 of plasma along with the buffy coat was aspirated and re-suspended and spun a second time. The resulting supernatant was removed and classified as PRP. Optimum clotting conditions was ascertained empirically. The resulting solutions were allowed to sit at room temperature and evaluated for clotting every 2min for the next 30min. Rat chondrosarcoma cells with a stable CAS9 red immunofluorescent marker (RCS-CAS9) were counted, suspended and introduced into 250ul of PRP at final concentration of 2 million, 1.5 million, 1 million, and 500 thousand cells/ml. The ideal cell concentration was determined by initiating clot formation with and then pipetting 10uL drops into a 96 well plate. Each clot was allowed to form and incubate for 2 days. Clots were evaluated for live cells and clot retraction. Bovine articular cartilage explants were harvested from the metacarpophalangeal joint an holes were drilled into the cartilage and top layer of bone to produce simulated osteochondral defects. Clotting was initiated and 5uL drops were introduced into each defect and allowed to clot. The defects were then covered with media and the explants incubated for 2 days. Each cartilage defect was then shaved off at the bone with a scalpel and evaluated under the microscope.

Results: Optimum clotting conditions were found to be 20mM final concentration of CaCl$_2$, at which full clot polymerization of the PRP was seen in a little over 10min. The optimum cell count was found to be 1 million cells/ml. Viewing the freestanding clots within the 96 well plate and within the osteochondral defect under phase light microscopy and via the immunofluorescence camera each showed a significant number of live cells with no clot retraction.

Discussion: PRP has been widely accepted and used clinically as a stimulator of tissue healing and regeneration. The ease at which it can be produced, and then the control at which polymerization can be generated makes it an excellent scaffold for future cell-based therapies. The results from this study prove that the clotting process can be initiated in PRP. The resulting solution can then be applied in a “drop-wise” fashion into an osteochondral defect, and allowed to clot within the defect with cells that remain viable over an extended period of time.

Significance: A viable scaffold made of autologous tissue is significantly more advantageous over various synthetic applications. While there are many other uses for PRP we have shown that it can also be used as a scaffold for future cell-based therapies for the treatment of osteoarthritis.
PET response criteria in solid tumors (PERCIST) predicts progression free survival, local control, and locoregional control whereas $SUV_{\text{max}}$ does not in early-stage non-small cell lung cancer treated with Cyber Knife Radiosurgery

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**Background:** Positron emission tomography with [$^{18}$F]-fluorodeoxyglucose integrated with computed tomography (FDG-PET/CT) is increasingly used as a standard of care in evaluating treatment response for patients diagnosed with early stage NSCLC. The results of the scan, including the $SUV_{\text{max}}$, are incorporated into the planning and follow-up stages of treatment and are evaluated by the PERCIST method to assess the tumor response after Cyber Knife Radiosurgery (CKRS).

**Objectives:** Analyze the importance of PERCIST criteria and of pre- and post-treatment $SUV_{\text{max}}$ from FDG-PET/CT imaging as prognostic elements for primary early stage NSCLC treated with CKRS.

**Methods:** 95 patients with primary stage IA/IB/IIA non-small cell lung cancer were included, only if they were not treated previously with radiation therapy for lung cancer. Patients received treatments in either 5, 4, or 3 fractions with doses such as 48 Gy, 50 Gy, 54 Gy, and 60 Gy with a median treatment time of 10 days. Local, locoregional, and distant failures were evaluated based on PET and CT imaging reviews. Overall-, progression-, local recurrence-, locoregional recurrence-, and distant metastasis-free survival were estimated by the Kaplan-Meier method from the MedCalc Statistical Software version 15.6.1. Univariate comparison of survival curves to determine whether $SUV_{\text{max}}$ and PERCIST criteria influenced outcomes was done by a Log rank test with MedCalc. Pre- and post-treatment $SUV_{\text{max}}$ were evaluated using cutoffs of $<5$ and $\geq 5$, $<4$ and $\geq 4$, and $<3$ and $\geq 3$. PERCIST 1.0 response evaluation method was used as described by Ding et al. (2014) with the following criteria: CMR, complete metabolic response; PMR, partial metabolic response; SMD, stable metabolic disease; and PMD, progressive metabolic disease (69, 14, 1, and 0 patients, respectively). This protocol was approved by the UMIRB.

**Results:** Median follow-up was 11.27 months in clinic and 9.23 months with PET or CT imaging. Median tumor size based on longest dimension in radiology report was 2.3 cm. Median overall survival and progression-free survival from the end of CKRS treatment was 15.43 and 11.89 months, respectively. PERCIST criteria predicted for progression-free survival ($p = 0.0392$), local control ($p = 0.0074$), and locoregional control ($p = 0.0151$) but did not achieve significance for overall survival ($p = 0.2065$) or distant control ($p = 0.9447$). Pre-treatment $SUV_{\text{max}}$ with a cutoff value of 5, 4, or 3 did not predict for OS, PFS, LC, LRC, or DC. Post-treatment $SUV_{\text{max}}$ with a cutoff value of 5, 4, or 3 did not predict for OS, PFS, LC, LRC, or DC.

**Conclusions:** PERCIST criteria is a statistically strong prognosticator of outcomes such as progression free survival, local control, and locoregional control in patients with early stage NSCLC treated with CKRS. These findings could support the use of additional treatment such as a wedge resection of the tumor if the initial PERCIST response is not as good as hoped for within a certain follow-up period. Pre- and post-treatment $SUV_{\text{max}}$ appears to not be a statistically significant indicator of outcomes for overall- and progression-free survival, local-, locoregional-, and distant-control.

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Background: Crossfit has gained popularity as a challenging strength and conditioning program utilizing gymnastics, weightlifting, and metabolic conditioning. Limited information is available about the risk of injury associated with Crossfit and how this risk may compare to other sports or activities. The purpose of this study is to determine the incidence of injuries sustained in Crossfit athletes during competition, and to determine risk factors associated with injury including exercise performed, level of fitness, level of competition, age, gender, height and weight. We theorized that the most common injuries would be musculoskeletal injuries of the shoulder, low back and knee and that the rates of injury would be higher than other sports because of the combined effects of weightlifting, gymnastics and endurance sports on Crossfit athletes.

Methods: Athlete exposure time, exposure events, competition level, exercises performed, age, gender, and injuries occurred were collected at multiple Crossfit competitions in Eastern North Carolina by a sports medicine physician. An internet search was used to try to obtain information not available at the time of competition. Injuries were reported per exposure hour and exposure event. Relative risk was calculated for gender.

Results: 562 athletes were observed in our study with 238 males, 318 female, 5 unknown gender. 125 athletes were scaled and 437 athletes were Rx’d. Ages ranged from 15 to 62 with a mean age of 30.6. A total of 13 injuries occurred for 293.88 exposure hours and 1,861 exposure events. 9 injuries were skin, 3 were musculoskeletal, and one was general medical. 1 injury occurred every 22.6 exposure hours and every 143 exposure event. By gender, male risk was 37.75 and female risk was 18.99 injuries per 1000 exposure hours. Making the relative risk for men 1.99. By competition level rx’d athlete risk was 48.87 injuries per 1,000 exposure hours. There was only one injury in a scaled athlete. Skin injuries were the most common at 69% of injuries and a rate of 1 injury per 32.65 exposure hours and 207 exposure events. There was only one msk injury per 97.96 exposure hours and 620 exposure events.

Conclusion: Skin injuries were the most common type of injury in our study with an incidence of 30.6 skin injuries per 1000 exposure hours. Compared to sports that make up components of Crossfit, skin injuries occurred at a higher than gymnastics (0.078 skin injuries per 1000 exposure hours\(^1\)) or weightlifting (0.035 skin injuries per 1000 exposure hours). This comparison would support our hypothesis that there is a combined effect on injuries on Crossfit athletes as compared to sports that are components of Crossfit. The lack of other types of injuries in our study makes comparing crossfit to other sports difficult but does imply that Crossfit is safer than previously theorized. Gender is a risk factor for injury and competition level is likely another risk factor. With rx’d men having the highest relative risk of injury. Future research could include a continuation of the current study to more clearly define incidence and risk factors. Research could also be conducted on general Crossfit participants exploring injuries occurred during daily training sessions, or on professional level functional fitness athletes exploring injuries occurring at the highest level of demand.

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\(^1\)Caine et al, A three-year epidemiological study of injuries affecting young female gymnasts, Physical therapy in sports 4(2003) 10-23

Investigation of CXCL1 and CCL2 Chemokines in Metastatic Sites of Murine Triple Negative Breast Cancer

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Tumor metastasis is a well-studied topic; however, the science of tumor cells homing to metastatic sites is still under investigation. The idea of a pro-metastatic niche in tumor metastasis has been proposed and is gaining acceptance. The pro-metastatic niche is a microenvironment that is formed in a secondary site that will be a host to tumor cells; chemokines and other growth factors that are secreted by cells at this site enhance the ability of the cancer cell to seed and grow. Based on previous studies, we hypothesize that CXCL1 (KC) facilitates migration of myeloid cells to the secondary site, which leads to the polarization of neutrophils and macrophages to N2/M2 pro-tumor phenotypes that express CCL2 (MCP-1). The purpose of this time course study was to investigate these chemokines in a mouse model of metastatic triple negative breast cancer. It has been shown that tissue metastasis in most mouse models are similar to human breast cancers with metastasis to the lungs, lymph nodes and liver. We studied the murine 2225L tumor, which shares characteristic of the human basal-like phenotype of triple negative breast cancer and which we have observed metastasizes to the lungs. Mice were implanted with 1-mm³ tumor chunks sc and euthanized at 5, 10, and 15 days post tumor implantation along with naïve healthy controls. One group was euthanized 30 days post tumor resection as a positive control for metastatic disease. At time of euthanasia, tissue at metastatic sites (lung, axial and inguinal lymph node, liver) as well as control tissue (kidney) were harvested. CXCL1 and CCL2 levels within tissue homogenates were measured by Enzyme Linked Immunosorbent Assay (ELISA). Our results show that both chemokines were significantly higher in lungs from mice with metastasis compared to lungs from naïve healthy mice. However, there were no significant differences in CXCL1 or CCL2 levels in lung, kidney, liver or lymph node samples from naïve and tumor-bearing mice 5-15 days post-tumor implantation. In earlier studies, our laboratory has detected these chemokines in lung tissue of mice with a greater 2225L tumor burden (i.e. at later time points post tumor implantation). Future experiments will include chemokine analysis and flow cytometry of infiltrating cells in tissues past the 15-day mark to characterize the pro-metastatic niche in triple negative breast cancer models.
Overexpression of Prognostic Factors in Papillary Thyroid Carcinoma with Lymph Node Metastasis

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Background: Papillary carcinoma of the thyroid (PCT) is a common malignancy with a number of molecular genetic associations. Evidence of how these molecular findings impact prognosis and treatment stratification is weak.

Objectives: To determine if tumors that present with lymph node metastases compared to tumors that do not present with lymph node metastases have significantly different molecular findings.

Methods: Immunohistochemical staining of seven different antibodies were performed using standard methods. Chi-squared tests will be used to determine statistical significance of variables comparing the two groups.

Results: Of the 73 patients we evaluated (37 with lymph node metastasis, 36 without lymph node metastasis), genetic marker expression was significantly different for the two groups. BRAF biomarkers demonstrated that 27/36 patients correlated with no expression and no metastasis, while 22/37 patients associated BRAF expression and lymph node metastasis (p=0.0043). In addition, p16 biomarkers revealed that 32/36 patients correlated with no expression and no metastasis, while 13/37 patients associated p16 expression and lymph node metastasis (p=0.0252). In contrast, the other five biomarkers (MET, PD-L1, MUC1, SPP1, CLIP2) were not statistically significant (p>0.05).

Conclusion: Expression of BRAF and p16 biomarkers were associated with the presence of lymph node metastasis in PCTs.
Enhanced recovery after surgery (ERAS) measures implemented on a high-volume surgical oncology unit decrease cost, length of stay (LOS), and mortality

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**Background:** ERAS protocols have been developed in order to decrease length of stay and cost in various clinical settings. Protocol measures focus on the perioperative period and aim to decrease surgery-induced stress, expedite the recovery of physiological function, and encourage early mobilization. However, it is unclear if improvement stems from the protocol itself or shifts in expectations.

**Objective:** We sought to analyze outcomes in a pilot ERAS study on a high volume surgical oncology unit to examine the effects of ERAS. We hypothesize that ERAS implementation by a surgical oncologist will decrease LOS and cost without compromising quality of care due to protocol measures rather than a change in expectations.

**Materials:** Introductory interdisciplinary educational seminars involving unit surgeons, residents, nurses, dieticians, and rehabilitation therapists were conducted. In order to validate our initial test of change, this protocol was adopted by one of three surgical oncologists with others serving as controls.

**Results:** A total of 394 patients undergoing elective abdominal surgery from June 2013-April 2015 were included. Median age was 63 years, a majority were female (51.8%), white (59.9%), had a Charlson comorbidity score of 0-2 (40.4%), and a Clavien complication grade of 0-I (63.2%). Implementation of ERAS resulted in a significant decrease in LOS (6.0 vs. 8.0 days; p= 0.016) and in-hospital mortality (0% vs. 2.9%; p= 0.033); the difference in cost ($21,674 vs. $25,994; p= 0.060) did not reach significance. Gender (p= 0.63), age (p= 0.36), race (p= 0.89), type of surgery (p= 0.49), comorbidities (p= 0.76), complications (p= 0.31), and readmission rates (p= 0.21) were similar. For the test surgeon, ERAS was associated with a decreased LOS (6.2 vs. 9.6 days, p= 0.024), cost ($21,674 vs. $30,380, p= 0.029), and mortality (0 vs. 3.3%, p= 0.044); differences in complications (grade II-V 32.2 vs. 42.6%, p= 0.064) and readmission rates (11.5 vs. 21.4%, p= 0.076) did not reach significance. For the control providers LOS, cost, mortality, readmission rates, and complications were similar before and after implementation of ERAS on this unit.

**Conclusions:** Full implementation of an ERAS protocol on a single high-volume surgical unit decreases cost, LOS, and mortality. This change can be attributed to protocol adherence rather than an ethereal change in unit culture.
Underutilization of Genetic Risk Evaluation and Testing in High Risk Breast Cancer Patients
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**Background:** BRCA1 or BRCA2 mutations account for most of the inherited breast cancer cases, and identification of these mutations helps guide treatment and risk reduction strategies. The National Comprehensive Cancer Network (NCCN) has set forth guidelines for referring patients at increased risk of hereditary cancer to genetic risk evaluation, counseling and testing if indicated. Studies have shown that providers do not consistently refer patients meeting criteria for genetic risk evaluation. Other barriers like race/ethnicity and socioeconomic factors (e.g. access, insurance, geographic location, education) have also been associated with underutilization of genetic services, especially in underserved populations.

**Objective:** We sought to assess the rates of referral for genetic risk evaluation among patients meeting criteria per NCCN guidelines, at Leo Jenkins Cancer Center (LJCC). We also sought to identify the potential barriers, to referral, counseling and testing among breast cancer patients at LJCC. A dedicated high risk breast cancer clinic was established in 2010 at LJCC, so we also analyzed rates of genetic referral, counseling and testing before and after 2010 at LJCC.

**Methods:** An IRB approved retrospective chart review of patients treated for breast cancer at LJCC between 2007 and 2014 was undertaken. Patients meeting NCCN criteria for genetic risk evaluation were identified; demographic, socioeconomic, and disease related information was obtained and analyzed to determine rates of referral, counseling and testing.

**Results:** From 2007 to 2014, 968 breast cancer patients were treated at LJCC and 46.2% (n=446) met NCCN criteria for further genetic risk evaluation. Of those who met criteria, only 59.6% (n=266) were referred and 45.7% (n=204/446) underwent genetic counseling. Reasons for not undergoing counseling after referral were largely undocumented (66.7%, n=41, p<0.0001). African Americans and Hispanics were more likely to meet criteria based on personal diagnosis alone. Patients from counties with low median household income were less likely to attend genetic counseling if referred (p=0.0209). Referral rate shows an increasing trend with time, although not significant: 2007-10 baseline (57.8%, n=121), and 2011-14 to genetic counselor at LJCC (64.4%, n=163). 81% (n=47) of patients with high probability of BRCA mutation underwent genetic testing, with financial reasons (81.8%, n=16) as the most commonly cited barrier for not being tested (p<0.0001).

**Conclusions:** Many patients in spite of meeting criteria for genetic risk evaluation were not referred for counseling thus depriving them of potential risk reductive strategies. Of those who were referred but did not undergo counseling, their reasons are largely unknown or undocumented, due to lack of follow up and communication. Socioeconomic factors were the most common barrier to genetic testing. We plan to develop an EMR automated alert and update system to help address the low referral and counseling rates.
Analysis of the Molecular Morphology of Mouse Heart by MALDI-TOF/MALDI-IMS

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Background: Matrix-assisted laser desorption ionization (MALDI)-imaging mass spectrometry (IMS) and MALDI-time of flight (TOF) allow acquisition of mass data for metabolites, lipids, peptides, and proteins directly from tissue sections with great spatial resolution. Importantly, MALDI-IMS/TOF provide high resolution imaging with spatial distribution of a molecule within a given biological tissue sample without prior knowledge of tissue composition and the use of antibodies.

Objectives: The purpose of this research was to determine if MALDI-IMS/TOF can be utilized to spatially locate the distribution of endogenous proteins and exogenous peptides within mouse heart tissues.

Methods: Two types of heart tissue samples were collected in conjunction with untreated controls and snap frozen in liquid nitrogen: 1) Target peptide (p1158/59 Matricryptin) was perfused systemically via a subcutaneous osmotic pump and heart tissue was harvested after 24 hours. 2) The left anterior descending coronary artery was ligated and infarcted non-reperfused heart tissue was harvested after 4 days to look for EphrinA1 expression. Each sample was sectioned using a cryostat, mounted on an indium tin oxide coated slide, washed, MALDI matrix applied using a TLC sprayer, spotted with standards (p1158/59 or EphrinA1), and analyzed using a Bruker Autoflex MALDI/TOF MS.

Results: For study 1, extracted ion images for control and peptide-treated heart tissue that corresponded to the targeted peptide standard were seen at an m/z 453.42 Da, 616.69 Da, and 677.93 Da. Regions with higher intensity were observed in treated heart tissue, compared to the control tissue, indicating a larger abundance in the treated tissue. For study 2, the extracted ion images for control and 4 day infarcted non-reperfused heart tissue indicated varying abundance of the ion at an m/z 24,275.65 Da that corresponded to the targeted protein. Both control tissue and non-infarcted tissue presented regions of higher intensity, indicative of an abundance of the targeted protein in specific spatial locations. Compared to the healthy regions, areas associated with infarct had a lower abundance of the ion indicated by a lower intensity of signal.

Conclusion: Higher signal intensity in the treated heart tissue compared to the control in study 1 suggests that systemic delivery of p1158/59 Matricryptin is a viable technique for cardiac treatment since the peptide homed to the myocardium. Moreover, p1158/59 levels could be detected and differentiated between tissues, which is supported by previous literature1. In study 2, a weak signal detected around infarcted areas when compared to the control tissue and areas of non-infarct suggests a decreased concentration of EphrinA1 in the infarct region, which is supported by previous studies2. These data suggest that MALDI-IMS/TOF has the ability to image heart tissue sections for the presence of EphrinA1 and p1158/59 Matricryptin. It should be noted that MALDI-IMS/TOF provides the ability not only to detect the primary analyte in question but also a range of potential novel analytes simultaneously that can assist in both future and retrospective studies.

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Viral transduction of human osteoarthritic cartilage in vitro and determination of transgene protein expression.

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**Background:** Osteoarthritis (OA) currently affects approximately 52.5 million US adults, and these numbers are estimated to rise to 67 million by 2030. In OA, articular cartilage lesions, induced traumatically or pathologically, lead to debilitating joint pain. Due to the fact that cartilage is avascular and aneural, repair of cartilage lesions does not occur efficiently. Genetically modified viruses are at the cutting edge of research as a possible treatment for OA. These viruses are genetically modified to produce proteins that inhibit cartilage degradation or increase chondrogenesis. Studies into which virus leads to the most efficient viral transduction, as well as which transgenes are the most efficient to promote cartilage repair, are at the forefront of cartilage research and are the focus of this study.

**Objectives:** We hypothesized that viral transduction and transgene expression would be more efficient by the smaller scAAV2 virus, as well as by chondrocytes in superficial zone cartilage versus the middle/deep layers of articular cartilage.

**Methods:** Viral transduction of thin superficial and middle/deep layer human OA cartilage slices from an 85 y.o. female and a 68 y.o. female in vitro used genetically modified Ad-ZsGreen-mycHAS2, scAAV2-EGFP, and AAV2-EGFP viruses. Following transduction for 72 hours at 37°C in DMEM/F12, cartilage slices were analyzed using fluorescence microscopy and western blotting. For western blots, lysates of the cartilage slices were prepared by freezing in liquid nitrogen, pulverizing tissue using a mortar/pestle system and a hammer, and dissolving in RIPA complete lysis buffer. Viral transduction was analyzed in the western blot by using anti-EGFP and anti-myc 1° antibodies and a donkey anti-mouse 2° antibody.

**Results:** Fluorescence microscopy analysis confirmed that direct viral transduction of cartilage cores and slices was possible. Transduction of superficial thin cartilage slices was more efficient than that of the middle layer. Also, it was found that the smaller scAAV2 virus yielded more transduction then that of the adenovirus or AAV2. Microscopy of cartilage slices transduced with AAV2 was nearly non-existent, which leads us to believe that AAV2 is not a good candidate for direct viral transduction of cartilage. Western blot analysis confirmed that it is possible to pulverize the cartilage using the method from this study and accurately detect proteins in the lysate. This also confirmed the transduction of the virus into the chondrocytes, as indicated by the positive detection of bands representing both the myc and EGFP transgenes.

**Conclusion:** This study yielded results that obtaining usable direct lysates from intact cartilage explants is feasible and, determined that there is transgene protein expressed in scAAV2-EGFP and Ad-ZsGreen-mycHAS2 transduced thin cartilage slices.
Acute C-spine injury can be accurately predicted in elderly patients presenting to the Emergency Department after a fall using a 4-point tool

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Background: In the rapidly growing elderly population, falls are a common reason for presentation to the ED. It is important for the provider to be able to rapidly, and accurately predict which of these patients are at risk for acute cervical spine injury. Existing clinical decision protocol use age ≥65 years to predict the need for a CT scan; however, the majority of these scans are negative. The development of a tool that can better predict if c-spine imaging is necessary in elderly fall victims would reduce costs, time, and radiation exposure to the patient.

Objective: To determine if specific criteria identified by a previous retrospective study of elderly patients presenting to the ED after a fall can accurately predict cervical spine injury prospectively.

Methods: A prospective, cohort study was performed using a convenience sample of patients ≥65 years of age, who present to the ED with a chief complaint of fall. The treating physician was asked to indicate the following: patient age, sex, high or low risk mechanism of injury, presence or absence of midline cervical spine tenderness, ability or in ability of the patient to rotate their neck 45°, independent living status, and whether they suspected a C-spine injury based on exam. The answers to these questions and the physicians’ suspicions were then compared to the radiology report noting the presence or absence of acute C-spine injury. Odds ratios, chi-square analysis; sensitivity and specificity were then calculated for the novel tool.

Results: Data was collected for 52 patients ranging between ages 65 and 100 (mean age = 83.8±8.8 years), with 4 acute cervical spine injuries discovered (7.6%). Our screening tool demonstrated 100.0% sensitivity and 45.8% specificity (TP=4, TN=22, FP=26, FN=0). High-risk mechanism (p=0.771), independent living status (p=0.064), physicians’ physical exam (p=0.809), midline cervical spine tenderness (p=0.001) and inability to rotate neck 45° (p=0.022) were all independently evaluated. A patient meeting all 4 of the criteria has a significant chance (OR=7.64) of having a C-spine fracture verified by CT scan. The existing Canadian C-spine Rule (CCR) had 100.0% sensitivity with 0.0% specificity, while CCR excluding age had 75% sensitivity (1/4 acute C-spine injuries missed) with 83.3% specificity. Of the 4 acute c-spine injuries discovered, all were living independently, 3 had midline tenderness, and 1 was unable to rotate their neck 45°.

Conclusion: Our data show that using the 4 factors previously identified as predictors for acute c-spine injury in elderly patients that present to the ED after a fall did in fact predict all positive CT findings, with the potential to reduce the amount of CT scans ordered by 42.3% when compared to CCR. It is possible to rule out acute c-spine injury and potentially reduce the need for CT scanning in this population. A continuation of this prospective study is needed to further define the role of all 4 criteria in the elderly population.
Evaluation of protein biomarkers to identify human testicular cancer subtypes

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Background: Testicular germ cell tumors (TGCTs) are categorized into seminoma (classical and spermatocytic) and non-seminoma,(embryonal carcinoma, yolk sac tumor, choriocarcinoma), or teratoma. The standard treatment for testicular cancer is a rather invasive, inguinal orchiectomy. A major therapeutic objective is to identify protein biomarkers in TGCTs to improve diagnosis and guide suitable treatment options. However, there are currently few identified biomarkers that are specific to the various types of TGCTs.

Objectives: The objective was to use immunohistochemistry to detect potential diagnostic and prognostic protein biomarkers in human normal and malignant testicular sections. We have focused on proteins involved in signaling pathways that regulate cell proliferation, differentiation, and apoptosis. We hypothesize that the localization and staining intensity of various markers of the Akt/PI3K pathway will be correlated with stages of germ cell development in normal tissue and with disease progression in malignant tissue.

Methods: Human testis samples were collected following orchiectomy, fixed overnight in 4% paraformaldehyde (PFA, pH 7.3), and embedded in paraffin. IHC was conducted on 5µm sections using a previously optimized protocol. Antibodies RPS6 (1:250, ProteinTech Group) and PCNA (1:500, ProteinTech Group) were diluted in blocking buffer and applied to sections, followed by a 1:500 dilution of biotinylated secondary goat anti-rabbit antibody. Immune complexes were stained using an ABC avidin–biotin–peroxidase kit (Vector Laboratories) and counterstained with hematoxylin. Sections were visualized with an Axio Observer A1 (Carl Zeiss microscopy, LLC) and images obtained with a Dage XL16 digital camera (Dage-MTI) #.

Results: Our preliminary results suggest that PCNA, a marker of proliferation, is detectable in seminoma. The identification of RARG, indicates critical role in development, reproduction, and organogenesis. While the detection of RPS6 is correlated with ribosomes and elongation factors necessary for translation.

Conclusions: Distribution and localization of protein biomarkers was detected through IHC in both seminoma and healthy tissue. The ability to screen Biomarkers has many potential applications in oncology, such as risk assessment, differential diagnosis, determination of prognosis, response to treatment, and monitoring of progression of disease.
2014-2015 MATCH Schools Demonstrate Variable Lesson Implementation Fidelity

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To address the childhood obesity epidemic, Motivating Adolescents with Technology to CHOOSE Health™ (MATCH) was designed in 2006 as a school-based wellness program to reduce obesity in rural schools. Previous research shows that implementing programs as intended increases program effects. MATCH includes up to 40 “core” and 71 “supplemental” lessons taught in the 7th grade. To promote better implementation in the 2014-2015 year, schools were encouraged to launch MATCH with a “wellness kick-off” unit of 13 lessons over a few weeks. For this study, we assessed: 1) program fidelity based on how well the kick-off unit and core content were provided, and 2) if the degree of fidelity was associated with student outcomes of decreased BMI z-score, signifying improved weight status. We hypothesized that, 1) degree of fidelity across schools would vary, and 2) children in schools with high vs low fidelity would demonstrate greater decreases in BMI z-scores (calculated from measured height and weight). Eleven eastern North Carolina middle schools and four South Carolina middle schools with high obesity prevalence participated in MATCH, with total N=1386 participants. Implementation fidelity variables (kick-off and core content) were assigned as High, Medium, and Low using specific criteria. Outcome measures included change in BMI z-scores from pre- to post-MATCH. Analyses conducted included Chi-square test for categorical data and GEE Parameter Estimates Analysis. Overall, implementation of the kick-off and core lessons varied, with only two schools having high kick-off fidelity, and three achieving high core lessons. There was no significant association between BMI z-score decrease and kick-off lesson fidelity, but interestingly there was a negative association between BMI z-score decrease and core lesson fidelity. The MATCH team is exploring details of lessons delivered and other program variables this year to further understand this result, and will be modifying teacher training to work to improve fidelity.
Patient Satisfaction Varies Among Acuity Levels in the Emergency Department

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Background: When evaluating patient satisfaction within Emergency Departments (EDs) across the country, a small percentage of patients are surveyed when discharged (i.e. lower acuity), using one generalized questionnaire. Surveys that are acuity specific, administered to patients who are both discharged or admitted provides data from a more representative population of ED patients and, potentially, a more accurate reflection of patient satisfaction.

Hypothesis: We hypothesized that patients of higher acuity levels would be more satisfied with their experience than those of lower acuity levels.

Methods: A prospective study using a convenience sample of ED patients was performed. Any patient able to provide informed consent was eligible for enrollment. Patients were surveyed in the Emergency Department of Vidant Medical Center before final disposition (discharge or admittance) using a set of standardized questions as well as questions tailored to the level of care required for differing acuity levels (1/2=highest acuity; 4/5=lowest acuity). Patients rated the specific aspects of their care on a 100 cm Visual Analog Scale with the 0 end of the scale representing the negative rating. Mean ratings for each question were compared across acuity levels using Analysis of Variance (ANOVA) or t-test where appropriate with p<0.05 indicating significance.

Results: Patients ratings of perceived severity of their health problem improved as severity level declined (acuity 1/2=34.3±3.9 cm; acuity 3=50.6±2.9 cm, acuity 4/5=74.3±4.4 cm; p<.001). Patients of acuity levels 4/5 reported that their ED visit made them feel significantly better than patients of acuity 1/2 and 3 (89.9±2.5 cm vs. 73.8±3.0 cm vs 71.8±3.9 cm, respectively; p<0.01). Patients of acuity levels 1/2 and 4/5 both felt their doctor and nurse were more considerate and attentive than patients of acuity level 3 (p<0.04). There were no significant differences among acuity levels when patients were asked if they received the treatments they felt were needed or how long it took to see a provider. Patients of acuity levels 4-5 felt that they understood their health condition/treatment plan significantly better than acuity 3 (92.2±4.1 vs. 75.0±3.8; p<0.001).

Conclusions: Although patients of higher acuity levels reported experiencing a more severe health issue, their satisfaction with their healthcare team and treatment were no different from the lowest acuity group, and higher than the intermediate acuity group. These results demonstrate that the degree of satisfaction with specific components of the ED visit may be significantly influenced by the acuity of the patient being surveyed.

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Pelvic Nerve Injury Reduces Bladder Smooth Muscle and Sympathetic Innervation in a Rat Model of Radical Hysterectomy

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Introduction: Hysterectomy is the most common non-obstetric surgery and approximately 40% of women 60 years of age or older have undergone the procedure. During radical hysterectomy (RH), the nerves controlling normal bladder function are routinely injured leading to lower urinary tract disorders such as incontinence, hypotonia and abnormal sensation which severely impact quality of life.

Objective: The aim of this study is to characterize changes in urinary bladder smooth muscle and autonomic innervation following bilateral pelvic nerve injury (BPNI), a model for RH. We hypothesized that following BPNI, smooth muscle area and nerve terminals expressing tyrosine hydroxylase (TH) and neuronal nitric oxide synthase (nNOS) would be decreased.

Methods: Female Sprague-Dawley rats (n=5/group), aged 12 weeks, underwent a modified “clock-crush” BPNI to simulate RH. Bladders were excised at 4 time points following injury (3, 7, 14 and 30 days). Sham animals underwent surgery in which the pelvic nerves were identified but not injured. Bladders were formalin fixed, paraffin embedded and sections were stained with Masson’s trichrome. Additional sections underwent immunofluorescence staining for alpha smooth muscle actin (aSMA), and nerve terminals expressing TH and nNOS. Nerve terminal counts were performed from 4 different areas on each bladder section by 2 blinded individuals.

Results: Nerve injured bladders showed significantly decreased smooth muscle area at all time points compared to sham bladders (S: 45%±4.1, 3d: 28%±1.0, 7d: 20%±2.7, 14d: 29%±2.1, 30d: 29%±1.1; p<0.005). Following BPNI, the number of nerve terminals expressing TH was significantly decreased in all injured animals compared to shams (S: 32±3.7, 3d: 13±5.8, 7d: 11±1.6, 14d: 11±1.0, 30d: 15±3.6; p<0.05). A trend towards a decrease in the number of nNOS nerve terminals was observed but was only significant at 30 days (S: 33±3.0, 3d:19±5.7, 7d: 23±6.2, 14d: 23±0.5, 30d: 15±1.2; p<0.05).

Conclusions: Pelvic nerve injury in a model of RH leads to marked decreases in bladder smooth muscle content and decline in sympathetic (TH positive) and nitrergic (nNOS positive) innervation. Future studies will also assess changes to cholinergic nerve input. These changes in bladder morphology and innervation suggest that following RH the inability of the bladder to properly void could be due to decreased smooth muscle and diminished autonomic innervation. Therapeutic strategies preventing the nerve injury mediated decline in smooth muscle and neuronal input may prevent the development of urinary incontinence and improve the quality of life of women undergoing RH.
Cognitive Maturation of a Longitudinal High School Population as Measured by a Computerized Baseline Neurocognitive Test

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Background Baseline computerized neurocognitive tests are commonly obtained preseason for an objective benchmark of individual neurocognitive function that can be used in the event of a concussion. However, given that the brain is still developing and cognitive maturation is taking place in an adolescent sample, establishing a valid baseline in high school athletes may be challenging\textsuperscript{1}. Therefore, an understanding of how cognitive maturation effects the normal nature and rate of change in the baseline test performance of an adolescent sample is needed.

Hypothesis The purpose of our study was to longitudinally track neurocognitive changes on baseline testing in a healthy high school population. Based on previous research, we hypothesized that there would be a significant improvement in neurocognitive test performance with increasing grade level.

Methods This study involved a retrospective chart review of 85 healthy high school athletes who completed four consecutive yearly baseline exams using Concussion Vital Signs online software prior to participation in athletics. One-way repeated-measures ANOVA were calculated between the consecutive raw score performance measures computed by Concussion Vital Signs to identify any grade level effects on performance.

Results There was a significant improvement between grade levels for the scores of psychomotor speed $F(3, 252) = 13.928, p = 0.000$, executive function $F(2.041, 171.434) = 79.238, p = 0.000$, cognitive flexibility $F(2.031, 170.639) = 77.912, p = 0.000$, reaction time $F(3, 252) = 10.994, p = 0.000$ and shifting attention correct response time $F(2.174, 182.6) = 21.027, p = 0.000$. Tukey’s Post hoc analysis showed significant improvement ($p < 0.05$) between all four years of high school for executive function only. Cognitive flexibility and shifting attention correct response time demonstrated significant improvement between the ninth and tenth grade as well as tenth and eleventh grade. Psychomotor speed and reaction time significantly improved between the ninth and tenth grade only.

Conclusions/Significance These findings support the hypothesis that high school aged athletes are undergoing cognitive maturation and that Concussion Vital Signs is sensitive to these improvements. This supports the need for annual baseline testing in these populations, rather than relying on a single baseline test during the first year of participation. These findings also suggest that simply using “return to baseline” cognitive performance as a benchmark of recovery in a cognitively maturing population may be inappropriate and that the effects of cognitive maturation should be taken into account when analyzing test performance in an adolescent sample.

EMERITAS Fluorometer detects concentrations of Troponin comparable to hospital values for ACS Positive patients.

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BACKGROUND: Troponin is the biomarker of choice for detecting cardiac damage in ACS patients. The EMERITAS Fluorometer, currently being evaluated for efficacy in detecting Troponin concentrations, is a device that provides a quicker result (~16 min.) that can be read by clinical staff at the bedside. This would provide convenience that would benefit clinical staff significantly. Currently, few studies have been conducted regarding the nature of the device and the Troponin results that it provides.

HYPOTHESIS: I hypothesize that the EMERITAS fluorometer detects concentrations of Troponin that are comparable to hospital values for similar time points in the course of ACS pathology and patient presentation.

METHODS: Samples of blood taken from ACS-positive patients at time points 0 hr, 2-4 hr, 6-9 hr and 12-24 hr were analyzed by the EMERITAS Fluorometer for both their Whole Blood and Plasma levels of Troponin. The concentrations of Troponin obtained from the EMERITAS device were then compared to hospital values at similar time points of patient presentation using bivariate plots.

RESULTS: Bivariate plots of Troponin values obtained from the EMERITAS device and the hospital values show significant correlation at time points 1 (R^2WB= 0.996, R^2PL=0.997), 2 (R^2WB, PL>0.999), and 3 (R^2WB, PL>0.999). The data, however, show a significant dissociation from Hospital Values at time point 4 (R^2WB=0.660, R^2PL=0.548). These data also imply a significant association between Whole Blood and Plasma levels of Troponin read by the EMERITAS device at the given time points.

CONCLUSIONS: Values of Troponin read by the EMERITAS Fluorometer show significant correlation with Hospital Values at time points 1-3, validating the hypothesis. This result implies that, for these points of patient presentation, the EMERITAS device provides a reliable analysis of a patient’s Troponin level result in an expedited time. At time point 4, however, the device shows a significant dissociation from the hospital values of Troponin. This is more than likely related to the differences between the hospital draw schedule and our own—the clinical staff usually drew earlier (closer to 12 hours) than the research group tended to (closer to 24 hours), catching the patient in a different stage of their pathology. This would account for the differences in Troponin values. The similarity of the R^2 values of Whole Blood and Plasma patient samples implies a significant association between the Troponin levels of these samples. This could be clinically relevant in consideration of which sample preparation may provide the most accurate result. From the results of this study, it appears that Whole Blood and Plasma samples serve as good indicators of blood Troponin concentration.
Docosahexaenoic acid Suppresses Influenza mRNA Expression and Improves the Inflammatory Profile in Lung Tissue

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Docosahexaenoic acid (DHA) is an omega-3 fatty acid found in marine fish oils. DHA is hypothesized to have anti-inflammatory effects; however, it is unknown if DHA can improve inflammation in diet-induced obesity, particularly in the context of influenza infection. This study aims to determine whether supplementing the diet with DHA can improve the inflammatory profile. Male C57BL/6 mice were fed one of the following three diets: control, high fat, or high fat diet supplemented with DHA (modeling human intake at \textasciitilde2.5 g/day). After 15 weeks of administering the diets, specific tissues were harvested to assess the inflammatory profile. Quantitative real-time PCR was used to compare relative mRNA expression of inflammatory and anti-inflammatory cytokines in lung and adipose tissue. The data revealed that DHA suppressed inflammatory TNF\textalpha and IL-6 cytokine expression in the lungs 7 days post-infection relative to the controls. Additionally, DHA increased anti-inflammatory cytokines (IFN\textbeta and IL-10) 7 days post infection and lowered mRNA levels of the influenza (PR8) virus. In adipose tissue, DHA did not exert any beneficial effects but did have a tendency to lower inflammatory cytokine expression prior to infection. The results suggest that DHA may have a protective effect against the influenza virus and rescue the inflammatory profile in the lungs in obese individuals. Further studies should be implemented to determine the clinical relevance of DHA.
National and Statewide Obesity Reports Underestimate the Prevalence of Obese 7th Grade Children in Eastern North Carolina

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Background: Amidst growing concerns for childhood and adolescent obesity, prevalence of the condition remains obscure in many regions of the United States; therefore, accurate regional prevalence estimates are needed.

Objective: To conduct an analysis of published data reporting the prevalence of childhood and adolescent overweight and obesity and compare these reports to data collected from the Motivating Adolescents with Technology to CHOOSE Health (MATCH) program in order to provide more accurate prevalence estimates of obese children and adolescents in eastern North Carolina.

Methods: Studies were collected through National Health and Nutrition Examination Survey (NHANES 2003-2012), Youth Risk Behavior Surveillance System (YRBSS 2003-2013), National Survey of Children’s Health (NSCH 2003, 2007, 2011/12), and the Child Health Assessment and Monitoring Program (CHAMP 2005-2011). Additional data was from the MATCH (2007-2014) program where samples of 7th grade children were selected from counties of eastern North Carolina.

Main Outcome and Measure: Prevalence of overweight (85th percentile ≤ BMI < 95th percentile for age and sex), obese (BMI ≥ 95th percentile), and severely obese (BMI ≥ 120% of the 95th percentile and/or BMI ≥ 99th percentile) children in eastern North Carolina based on MATCH data.

Results: MATCH cohorts showed higher prevalence of overweight and obese children enrolled in middle school aged classrooms. Prevalence of children with a BMI greater than or equal to the 85th percentile was approximately 50% in each MATCH cohort from 2007-2014; whereas, NHANES reports approximately 33% for a similar age and time. Though the prevalence of overweight children was similar between studies, MATCH showed higher prevalence of obese and severely obese children by approximately 150% and 200% respectively. These trends are consistent across other statewide studies.

Conclusion: Compared to existing prevalence reports, the prevalence of overweight and obese 7th graders in eastern North Carolina is almost double. This discrepancy may be due to underreporting from utilizing telephone surveys, to under-sampling, and/or selection bias within studies. What is clear is the prevalence of overweight and obese children in MATCH cohorts is much higher than the national and statewide average reports. Future studies will address discrepancies between gender and race to identify populations with highest prevalence and with most severe risk of developing obesity and its comorbidities.
Experience with Dental Emergency Department Visits

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Background: Visits to the emergency department (ED) for dental problems are on the rise. Often times these visits offer no definitive treatment and would be better treated in a dental office.

Objective: The purpose of the study was to evaluate dental ED visit data from Vidant Hospital and experience with dental ED visits of patients at ECU School of Dental Medicine (SoDM) clinics at Ross Hall. This descriptive data will be used to later evaluate the impact of the newly opened ECU SoDM clinics at Ross Hall on dental ED visits at Vidant Hospital.

Methods: This two part study consisted of 1.) A retrospective analysis of Vidant Hospital ED records and 2.) A questionnaire dispensed at the Advance Care Clinic at ECU SoDM.

Results: There were 6,114 dental ED visits at Vidant Hospital, accounting for 1.90% of ED visits from 01/2011 to 05/2015. The percent of dental ED visits compared to all ED visits decreased from 1.96% in 2011 to 1.71% in 2015. Monthly progressions show that dental ED visits in 2015 are lower than previous years, with the exception of January. Three diagnostic codes accounted for 91.2% of dental ED visits. Unspecified codes accounted for 77.7% of dental ED visits, indicating no definitive diagnosis. According to the patient questionnaire, 16% of patients had a prior dental ED visit, 48% had no dentist, 47% presented with a toothache, and only 7% received dental treatments.

Conclusion: Since opening clinics at Ross Hall in 2013, visits have continued to increase. At the same time, dental-related ED visits visually appear to be decreasing. Patients at Ross Hall that reported having a dental-related ED visit showed that the primary reason for dental ED use was related to not having a dentist. The questionnaire also reported that the major reason for ED use was a toothache and the most common treatment was pain pills. Further research will be conducted to evaluate the impact of ECU SoDM clinics at Ross Hall on dental ED visits at Vidant Hospital.
Novel nomogram combining depth of invasion and size can accurately predict the benefit of regional lymphadenectomy for appendiceal neuroendocrine tumors (A-NET)

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**Introduction:** The need for regional lymphadenectomy in A-NET is predicated on the risk for nodal metastasis. Although depth of invasion is predictive of nodal metastases in other gastrointestinal NET, current guideline recommendations for A-NET are based solely upon size.

**Hypothesis:** The purpose of this study is to determine whether depth of invasion, in combination with size, accurately predicts the risk of nodal metastasis in A-NET.

**Methods:** Patients with A-NET from 1988-2012 were identified within the SEER registry. Depth of invasion was defined as limited to lamina propria (LP), invading or through muscularis propria (MP/TMP), or through serosa (TS).

**Results:** A total of 1,024 patients were included, with a majority being female (55.7%), white (86.8%) and node-negative (81.1%). On univariate analysis, risk of nodal metastasis was associated with size (<1 cm, 1.9%, 1-2 cm, 19.7%, 2-4 cm, 32.5%, and >4 cm, 37.2%; \(p<0.001\)), depth of invasion (LP 4.1%, MP/TMP 17.4%, TS 44.3%; \(p<0.001\)), and extent of surgery (appendectomy 8.7% vs. colectomy 31.9%; \(p<0.0001\)). On multivariate analysis, size (<1 cm vs. 1-2 cm OR 8.80, <1 cm vs. 2-4 cm OR 15.68, <1 cm vs. >4 cm OR 15.16; \(p<0.001\)), depth of invasion (LP vs TS OR 6.67; \(p<0.0001\)), and extent of surgery (appendectomy vs. colectomy OR 2.98; \(p<0.0001\)) continued to be associated with nodal involvement. When only patients with colectomy were considered, results were similar on both univariate and multivariate analyses.

On univariate survival analysis, size (<1 cm, 95.6%; 1-2 cm, 94.9%; 2-4 cm, 88.4%; and > 4 cm, 73.7% 5-year disease-specific survival (5-y DSS), \(p<0.0001\)), depth of invasion (LP 96.9%, MP/TMP 93.2%, TS 77.1% 5-y DSS, \(p=<0.001\)), extent of surgery (appendectomy 94.4% vs. colectomy 87.1%, \(p=0.0002\)), and N stage (N0 94.7% vs. N1 77.7%) predicted survival. In a Cox regression model, extent of surgery and N stage continued to predict survival. A nomogram (Figure 1) was created to predict the risk of nodal metastases. The model accurately determined the likelihood of lymph node metastasis, with an area under the receiver operating characteristic (ROC) curve of 0.83.

**Conclusion:** This simple nomogram, which incorporates size and tumor extension accurately predicts the risk for regional nodal metastases in A-NET. In addition to providing valuable information on risk for regional nodal metastases, depth of invasion independently predicts survival.
Multiple sclerosis (MS) is an inflammatory disease that causes demyelination in CNS white matter and is considered to be an autoimmune disease instigated by self-reactive CD4⁺ T cells. Current treatments include the long-term use of immunosuppressive and immunomodulatory drugs, which can have severe side effects prompting the need for more effective therapies, such as tolerogenic vaccines. The development of tolerogenic vaccines depends upon our understanding of infectious tolerance, which is defined as a process whereby regulatory T cells (Tregs) impart their suppressive capabilities onto naïve T cells. We hypothesized that MHC class II on Tregs facilitates infectious tolerance and that the presence of an MHC II blocking antibody, Y3P, will disrupt this mechanism.

Splenocytes from CD45.2 2D2-FIG mice, whose T cell repertoires are specific for myelin oligodendrocyte glycoprotein (MOG35-55) and possess a fluorescent Treg reporter, were activated with MOG and TGF-β to generate MOG-specific Tregs. These MOG-specific Tregs (stimulator Tregs) were then added to a culture of naïve CD45.1 2D2-FIG responders in the presence or absence of MOG. Infectious tolerance was measured by the expression of FoxP3 in the CD45.1⁺ population (responder T cells). Concurrent characterization of cell surface proteins found on CD45.2⁺ stimulator and CD45.1⁺ responder Tregs, such as MHC class II, CTLA4, and PD-1, was done by use of flow cytometry.

This experimental system is unique in the field as it allows the measurement of infectious tolerance in an in vitro system, in that as many as 60% of the FOXP3null CD45.1 responder T cells converted to the FOXP3⁺ Treg phenotype during co-culture. This system thereby allowed an assessment of the conditions necessary for Treg recruitment of naïve T cells into the Treg lineage. This analysis showed that CD45.2 stimulator Tregs were more efficient than CD45.2 Tconv for FOXP3 induction in CD45.1 responder T cells. Contrary to expectations, infectious tolerance was not dependent upon antigen recognition. In fact, infectious tolerance was blocked by antigen recognition. That is, the specific antigen MOG35-55 inhibited infectious tolerance, and the anti-MHCII monoclonal antibody that blocked antigen recognition substantially augmented infectious tolerance. Our interpretation is that antigen presentation by professional APC facilitated differentiation of Tconv at the expense of Tregs, and that blockade of antigen presentation facilitated differentiation of Tregs at the expense of Tconv subsets. These data provide suggestive evidence that professional APC such as DC favor Tconv subsets, and that other undefined APC subsets favor Treg development. These data reveal an immunological default whereby Tregs recruit naïve T cells into the Treg lineage as a basic feature of immune homeostasis.
Reduced Idebenone and Idebenone-linked compounds deliver electrons to cytochrome c independent of complexes I, II and III in isolated rat heart mitochondria in the absence of substrates.

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Background: There are more than 50 heritable mitochondrial diseases of metabolism known. One in every four thousand children in the United States will be afflicted with some form of mitochondrial disease by age ten (3). Idebenone has been tested in a number of clinical trials to treat diseases such as: Friedrich’s Ataxia, Duchenne Muscular Dystrophy, Leber’s Hereditary Optic Neuropathy, Leigh Syndrome, Alzheimer’s and even Huntington’s disease (1,2). Similar in structure to CoQ10 its mechanism of action is thought to mostly be through complex III, but there is still much to learn about the efficacy and utility of Idebenone (1,2).

Hypothesis: Idebenone in the reduced form (idebenol) and SBT 60, 61 (reduced Idebenone-linked compounds) will feed electrons into complex III of the ETC and allow respiration to occur in the absence of complex I and II activity.

Methods: Hearts and heart mitochondria (LV only) were isolated from male Sprague Dawley rats purchased from Charles River. Before Isolation the right ventricle and atria were removed so the sample represented left ventricular mitochondria only. Respirometry of Isolated mitochondria was measured using Oroboros O2k respirometers. Langendorf studies were done using whole hearts put into ischemia for 20 minutes and then reperfused with 1uM of each compound incorporated into normal oxygenated Krebs buffer. Additionally, spectrophotometric assays analyzing reduction of cytochrome c.

Results: In order for Idebenone to significantly increase the respiration of isolated mitochondria, it must first be reduced to Idebenol. Each compound was studied at a concentration of 10, 1 and 0.1uM and was found to exhibit a statistically significant increase in respiration in a dose dependent manner. A one way ANOVA was performed with Bonferroni correction with p values < 0.05 to determine statistical significance. In the non-reduced form, these compounds demonstrated cardioprotective effects significantly reducing infarct size at a concentration of 1uM. Additionally, spectrophotometric assays showed statistically significant increases in the level of reduced cytochrome c in the presence of 100uM of each compound in the reduced form but very little in the oxidized.

Conclusions/Significance: These findings support the hypothesis that Idebenone must first be reduced in order to supply the ETC with a significant amount of electrons. However, contrary to previous studies (1), our data suggest that Idebenol has the ability to donate electrons directly to cytochrome c without the necessity of complex III activity.
