Abstract
The purpose of this study was to quantify the levels growth factors (PDGF-AB, VEGF, IGF-1, TGF-β1) within bone samples at the fusion site of diabetic patients undergoing hindfoot fusion to determine any correlation with successful fusion.

Methods
The study included 10 adult diabetic subjects from one U.S. center, with an average age of 57.9 years (range 49-71). Interventions included hindfoot fusions with fixator (n=8) and pantalar fusions with fixator (n=2). During each procedure, a bony bed sample was taken from the fusion site, and frozen. This local bone bed was analyzed for growth factors using ELISA kits. A PRP concentration was applied to each fusion site during the procedures.

Results
Three of the ten patients sustained a non-union outcome. After normalizing to BCA total protein levels, a 70% decrease in PDGF-AB (0.044 pg/µg non-union to 0.149 pg/µg union; p=0.016) and a 44% decrease in VEGF (0.522 pg/µg non-union to 0.924 pg/µg union; p=0.031) was observed in the three non-union bone samples compared to the fusion group. No difference existed in the levels of IGF-I between the groups. Insignificantly higher levels of TGF-β1 were detected in the non-union group (0.199ng/µg non-union to 0.142ng/µg union; p=0.544).

Conclusions
Differences in local growth factor levels exist between DM and non-DM patients’ bone. Potentially, growth factor levels (PDGF-AB, VEGF) in bone may affect the outcome of successful arthrodesis in diabetic patients. This study supports the concept that growth factor levels within the fusion site of a diabetic patient may affect the outcome of a successful hindfoot fusion.

Keywords
platelet rich plasma, diabetic fracture non-union, growth factor

Discussion
The objective of this study was to compare levels of growth factors within bone samples at hindfoot fusion sites of diabetic patients who achieve union and non-union. Four growth factors (PDGF-AB, VEGF, IGF-1, and TGF-β1) were measured to ascertain any correlation with successful union. These growth factors serve an integral role in the bone healing process (19). This study consisted of ten adult DM patients requiring hindfoot fusion. During each procedure, a PRP concentration was applied to each fusion site. These procedures resulted in seven unions and three non-unions among the ten patients. Quantifying the levels of growth factors of interest, after normalizing to BCA total protein levels, found that the four non-union samples had a 70% decrease in PDGF, a 44% decrease in VEGF, no change in IGF-I, and a non-significant increase in TGF-β1. These results confirm our hypothesis that certain growth factor levels within the fusion site of DM patients have an impact upon the success of the hindfoot fusion.

Several clinical studies demonstrate that high risk patients have significant growth factor deficiencies at the bone healing site. Street et al. showed that elderly patients had a decreased baseline level of growth factors as compared to younger individuals (20). Giannoudis et al. indicated that wide baseline growth factor level variations exist amongst patients ranging from all ages from both genders (21). This could explain why some patients in our study were able to achieve union and others resulted in non-union following PRP application. Tyndall et al. demonstrated significantly lower levels of PDGF using a DM BB Wistar rat fracture model, correlating to impaired osseous healing (22). This data was corroborated by the present study in humans, which demonstrated a 70% decrease in PDGF-AB levels from the bony bed in patients with non-unions. In DM BB Wistar rats, Gandhi et al. showed a reduction in protein levels of PDGF-
AB, VEGF, IGF-1, and TGF-β1 (23). The current study demonstrated complementary findings that patients, who proceeded to a non-union, exhibited a 44% decrease in VEGF. However, our study demonstrated no significant differences in local IGF-1 or TGF-β1 levels in diabetic patients with non-unions.

Levels of intrinsic growth factors present at the site of injury have been shown to be critical in appropriate fracture healing. Several growth factors produced locally within the fracture microenvironment play critical roles in cellular chemotaxis, cellular proliferation, extra-cellular matrix production and angiogenesis. Tyndall correlated decreased levels of PDGF with impaired fracture healing of diabetic BB Wistar rats (22). Street, specifically showed the reduced levels of PDGF in fracture hematomas of the elderly compared to fracture hematomas of people of younger age (20). A study by Gandhi et al. demonstrated that two of these growth factors PDGF and TGF-β have significantly decreased concentrations within the fracture hematoma in diabetic individuals, compared to non-diabetics. Gandhi found levels of these growth factors in plasma were significantly elevated in diabetic individuals compared to non-diabetics, supporting the concept that these factors are absent in the local microenvironment of early diabetic fracture healing (24). This finding is corroborated by the present study. Street et al. further demonstrated that elderly patients had a lower baseline level of VEGF as compared to younger individuals, while requiring a higher baseline levels for an appropriate angiogenic response (20). With an average age of approximately 50 years, non-union patients enrolled in the current study displayed decreased VEGF levels. This supports the concept that elderly fracture healing is highly dependent on local intrinsic growth factor concentrations within the callus microenvironment.

Impaired osseous healing following elective arthrodesis has been a common observation in the DM population. Elective arthrodesis in patients with DM has been analyzed in several series, with higher complication rates compared to their non-DM cohort. Papa et al. indicated that diabetic patients have a greater period to union following foot and ankle surgery with increased complications, ranging from superficial wound infections, to osteomyelitis and wound slough (6). This sentiment was echoed by Stuart et al., who showed that ankle arthrodesis in diabetic patients leads to a high failure rate with many complications (7). Since all the patients in current study were diabetic, complications and non-unions can be attributed to decreased levels of growth factors at the bone bed. This finding is supported by our previous study (23).

Application of PRP has been shown to be successful in increasing union rates of diabetic patients undergoing foot and ankle surgery. Bibbo et al. demonstrated that diabetic patients, who were not given growth factors, displayed a longer period to union or sustained a non-union (25). Grant et al. demonstrated that PRP administration resulted in an increased number of solid fusions among diabetic patients undergoing Charcot’s foot reconstruction (26). Our series tried to overcome the systemic disease of DM upon bone healing by adding PRP adjunct to the fusion site of all patients. All patients were given a concentrated growth factor dose in the form of platelet rich plasma (PRP) to provide the best possible chance of union. Unfortunately, three out of the ten patients still resulted in non-union.

The strength of this study lies within several parameters. All of the patients had similar diagnoses as well as the same bone preparation prior to samples being taken. The samples were all taken from cancellous bone within the surgical site. Recent studies have demonstrated a local increase in growth factor levels (PDGF, VEGF) following reaming of femoral canals during fracture fixation (22). Although our samples were not taken from fracture sites, we would expect a similar increase in local growth factor levels at the surgical site from the bed preparation released locally during surgery. While having a control sample from non-surgical site bone would help provide further insight into our study, the procedure to obtain such a sample would cause similar trauma and we feel it was not a reasonable addition to the initial surgery.

One of the potential weaknesses of this study is that two-thirds of the non-unions were from the pantalar fusion group. Pantalar fusions are intrinsically more difficult and have a higher complication rate compared to ankle fusions (27-31). In one study of pantalar and tibiotalocalcaneal fusions, 23 of 27 feet went into solid union (14 pantalar fusions and 13 tibiotalocalcaneal fusions), and all four of the nonunions were from a single joint in the pantalar group (32). This demonstrates the decreased success rate in pantalar fusions. However, the small sample size from our study limits our ability to compare success rates to the current literature.
Further work can be done to augment this study with additional parameters such as administering SF-12 Health Status Surveys, Foot Pain Disability Index, and an AOFAS scale. Another improvement would be to increase sample size for an improved statistical analysis. Additionally, CT scans could be taken to more accurately determine the healing status of patients. At the time of this study, radiographs were considered the gold standard in tracking a patient’s healing progress. Recent publications have shown that CT scans are more accurate in determining healing status of patients, and therefore, for future studies CT scans should be acquired. Finally, the effects of other risk factors’ upon DM osseous healing, such as smoking, may be considered for future investigation.

In conclusion, the results of our preliminary study suggest that DM patients with non-unions, after undergoing hindfoot or pantalar fusions with external fixation, have decreased levels of growth factors, specifically PDGF-AB and VEGF, when compared to patients who achieve union. Our results imply that local growth factors, such as PDGF-AB and VEGF, may play an integral role in the early bone healing.


