ORIGINAL ARTICLE

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Patient Body Mass Index Has No Direct Effect on The Characteristics of Primary Tenocytes Derived from Torn Rotator Cuffs

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ABBREVIATIONS
ALP: Alkaline phosphatase
BMI: Body mass index
DMEM/ F-12: Dulbecco's modified Eagle's Medium: Nutrient Mixture F-12
ECM: Extracellular matrix
FBS: Fetal bovine serum
IGF-1: Insulin-like growth factor
PDGF: platelet-derived growth factor
TGF- β1: transforming growth factor-β1

SUMMARY

Background. Obesity is associated with an increased risk of rotator cuff tears and impaired tendon healing after surgery. This study aimed: 1) to investigate the influence of patient body mass index (BMI) on cellular function in tenocytes derived from diseased torn human rotator cuff; 2) to determine if BMI altered the response of tenocytes to tenogenic growth factors.

Methods. Tenocytes were isolated from torn supraspinatus tendons of patients undergoing rotator cuff surgery. Tenocyte growth and collagen production were determined by alamarBlue and Sirius red assays, respectively, at baseline and in the presence of IGF-1, TGF- β and PDGF after 72 hours. Changes in the relative expression of genes important in tenocyte, chondrocyte and osteoblast biology were determined using real-time PCR. Correlation analyses were performed between patient BMI and tenocyte function and gene expression.

Results. BMI had no direct effect on tenocytes with no significant correlations between patient BMI and cellular behaviour, gene expression or response to growth factor treatment. Higher cellular growth and collagen production were observed in response to PDGF and TGFβ treatment, while IGF-1 had minimal effect. TGF-β was associated with higher expression of tenocyte-related genes, collagen Iα and scleraxis, while PDGF resulted in higher expression of adipose marker, *PPAR*-γ, suggesting it may be promoting de-differentiation from a tenocytic phenotype.

Conclusions. In summary, BMI does not influence tenocyte growth, collagen synthesis or gene expression profile *ex vivo*. These findings have significant clinical implications, as they suggest that growth factor treatment will be effective in patients independent of BMI.

KEY WORDS
Obesity; tendon; growth factors; healing; rotator cuff.

INTRODUCTION

Rotator cuff tendon tears are a common cause of shoulder pain and disability. With an increasingly active, ageing population, the demand for rotator cuff surgeries is rising (1–3). Despite recent advances in surgical technique, rates of post-operative re-tear remain high, and failed repairs result in persistent pain and loss of function (4–6). Surgical failure occurs mainly as a consequence of inadequate healing at the tendon-bone interface (7, 8).

Recently, obesity has been investigated as a risk factor for impaired rotator cuff healing. Several clinical studies have identified obesity as an independent negative risk factor for the occurrence and severity of rotator cuff tears (9–12) and reported a negative correlation between obesity and impaired tendon healing response (13–15). Body mass index (BMI) is associated with higher re-tear rates after rotator cuff surgery and lower post-operative functional scores (16, 17). Despite obesity emerging as a key and a potentially modifiable risk factor for poor tendon healing, few studies have investigated its effects on tendon at the cellular level (9, 13, 16, 17).

Over the past decades, orthopaedic research has focused on using growth factors to biologically augment tendon healing with promising results (18–20). Several growth factors have shown success in pre-clinical studies by promoting rotator cuff healing by increasing cellular recruitment, proliferation, differentiation, extracellular matrix (ECM) synthesis, and remodelling (21–23). In obese patients, high levels of pro-inflammatory cytokines and high mechanical load promote chronic low-grade inflammation that influences the activities of various mesenchymal cells, including tenocytes (13, 14, 24–26). For this reason, tenocytes derived from obese patients may exhibit differential cellular phenotypes and respond differently to growth factor therapies. A better understanding of these mechanisms may help to develop more effective therapeutic interventions that could reduce the musculoskeletal sequelae of obesity.

We hypothesised that 1) patient BMI would be correlated with negatively altered tenocyte phenotype; 2) patient BMI would reduce the effects of growth factor treatments on tenocyte function and gene expression. The aims of this study were: 1) to investigate how patient BMI influences cellular phenotype in tenocytes derived from diseased torn human rotator cuff; 2) to determine if BMI altered the response of tenocytes to growth factors insulin-like growth factor (IGF-1), transforming growth factor-β1 (TGF-β1) and platelet-derived growth factor (PDGF).

METHODS

Reagents and ethical approval

Dulbecco's modified Eagle's Medium: Nutrient Mixture F-12 (DMEM/F-12), penicillin-streptomycin mixture (10,000 U/mL), and fetal bovine serum (FBS) were obtained from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). Bovine serum albumin (BSA) was obtained from Immuno-Chemical Products Ltd. (Auckland, New Zealand). IGF-1, PDGF and TGF-β1 were sourced from Sigma Aldrich (St Louis, MO, USA).

For human tenocytes, human sample collection was approved by the New Zealand Ministry of Health Northern Regional Ethics Committee (NTX/05/06/058/AM14 - Date of approval: March 11, 2020). All participants provided written informed consent for collection of samples. All participants were undergoing arthroscopic supraspinatus rotator cuff repair, had supraspinatus tendon tears and were symptomatic prior to surgery. Demographic details on age, gender and ethnicity and biomorphic measurements on height (cm), weight (kg) and BMI (kg/m^2) were collected. Tendon samples were divided into samples from healthy-weight and overweight patients (BMI < 30 g/m^2), and those from obese patients with a high BMI (≥ 30 kg/m^2) for a sub-group analysis (27). Because tear size has been correlated with progression of pathology with reduced likelihood of repair with chronic disease, tear sizes were classified as follows: small (≤ 1 cm), medium (> 1 and ≤ 3 cm), large (> 3 and ≤ 5 cm) and massive (> 5 cm in anterior-posterior length) (28, 29). Patients were excluded from the study if they were: < 18 years; had previous shoulder surgery; or had other shoulder pathology, rheumatoid arthritis or other systemic inflammatory diseases.

Primary tenocyte cell culture

Primary tenocyte cell culture was performed, as previously described (30). Briefly, excess tissue from patients undergoing rotator cuff repair surgery was kept hydrated at 4 °C until use. Supraspinatus tendon tissue was roughly cut into pieces smaller than 0.5 cm^2 and digested in 0.5 mg/ml of dispase (Sigma-Aldrich) and 0.5 mg/mL of collagenase (Sigma-Aldrich) in DMEM/F-12 with 10% FBS at 37 °C for up to 18 hours until all ECM had been digested. The cell suspension was then passed through a cell strainer, washed, and re-suspended in enzyme-free media. Cells were cultured in DMEM/F-12 with 10% FBS in 75 cm^2 flasks (Corning, Corning, NY, USA) and incubated at 37 °C with 5% carbon dioxide until confluent. Cell cultures were frozen down in liquid nitrogen before being used for these experiments.

Cell viability assays

As described previously (30), primary human tenocytes were seeded in 24-well plates (Greiner BioOne, Kremsmünster, Upper Austria), at a density of 2.5 × 10^4 cells/well and cultured in DMEM: F-12 with 5% FBS for 24 hours. After this, the cell media was changed to growth arresting media DMEM: F-12 with 1% FBS. Following 24 hours of incuba-

tion, initial cell growth was measured by adding alamarBlue (Life Technologies, ThermoFisher Scientific) at 5% of final concentration into each well for 4 hours at 37 °C. Then, 200 µl of the alamarBlue conditioned medium was transferred to a 96-well plate (Greiner Bio-One), and fluorescence (excitation 540 nm; emission 630 nm) was read using a Synergy 2 multi-detection microplate reader (BioTek Instruments Inc., Winooski, VT, USA). Cells were then incubated with fresh growth arresting media with treatment compounds PDFG (20 ng/mL), TGF-β (20 ng/mL), and IGF-1 (100 ng/mL). These concentrations of growth factors have previously been demonstrated to have tenogenic effects (31). Cell growth was then re-measured using alamarBlue after 72 hours, as above. To report baseline function, the change in alamarBlue was expressed as a ratio of the untreated baseline measurement after 24 hours in growth arresting media and the repeated readings after 72 hours exposure to the treatment growth factor. There were four wells in each treatment group. To report function in response to growth factors, the change in alamarBlue in the treatment group was expressed as a ratio of the untreated control fluorescence readings.

Collagen deposition assays

Tenocytes were seeded in 24-well plates, at a density of 7.5 × 10^4 cells/well, and cultured in DMEM/F-12 with 5% FBS for 24 hours, as previously described (30). Cells were then incubated in fresh DMEM/F-12 1% FBS and 50 µg/ml AA2P with the same treatment compounds as above. After 72 hours, cells were fixed with Bouin's solution (71% saturated picric acid, 24% pure formalin, 5% 0.5 M acetic acid) for 30 minutes and then stained with 0.1% Sirius red dissolved in saturated picric acid for 1 hour. At the end of this incubation, cells were washed with 0.01 M hydrochloric acid five times and left to air dry. The dye was released using 0.1 M sodium hydroxide, and 200 µl of the released dye was transferred to a 96-well plate. Absorbance was measured at 570 nm using a Synergy 2 multi-detection microplate reader. There were four wells in each treatment group.

Gene expression analysis

Gene expression analysis was performed at two experimental time points: firstly following the initial culture and isolation of tenocytes for baseline comparisons, and secondly, after cells were cultured treated as described above under cell viability assays. Analysis of gene expression was performed using a previously established protocol (31). Total cellular RNA was extracted from cultured cells and purified using the RNeasy minikit (Qiagen, Venlo, The Netherlands). Genomic DNA was removed using RNase-free DNase set (Qiagen). The quality and concentration of the extracted RNA were measured using NanoDrop Lite Spectrometer (Thermo-Fisher, Victoria, Australia). Complementary-DNA was prepared by using 500 ng of RNA with super-script-III (Life Technologies, Carlsbad, CA, USA). Primer-probe sets were purchased as TaqMan Gene Expression Assays (Life technologies). The multiplex polymerase chain reaction was performed with FAM specific for genes of interest (alkaline phosphatase (*ALP*), collagen Iα1, scleraxis, *SOX-9*, *RUNX-2, PPAR*-γ) and VIC-labelled 18S endogenous ribosomal RNA probes, according to the manufacturer's instructions, using an ABI PRISM 7900HT sequence detection system (Applied Biosystems, Foster City, CA, USA). Samples were assayed in triplicate. The ΔΔCt calculation method (32) was used to determine the relative level of messenger RNA expression, normalised to the values of the non-treated cells (control).

Statistical analysis

All results are shown as mean ± standard error of the mean (SEM). Statistical analysis was performed using a one-way analysis of variance (ANOVA) test with Dunnett's *post-hoc* analysis. Pearson r test was used for analysing the correlation between patient BMI with tenocyte cell growth, collagen deposition and relative gene expression. A Mann-Whitney U test was used to compare patients with BMI < 30 kg/m^2 with BMI ≥ 30 kg/m^2. A P-value of < 0.05 was considered statistically significant. A false discovery rate (FDR)-adjusted P-value for correlation analyses between BMI and relative gene expressions level was calculated, as previously described (33), to control for the expected proportion of falsely rejected hypotheses. All statistical analysis was performed using the GraphPad Prism 8 software (GraphPad Software, San Diego, California).

RESULTS

Patient Demographics and Biomorphic Measurements

The study group consisted of 13 supraspinatus tendon specimens from 13 patients, 5 women and 8 men, with a mean age of 59.1 ± 2.81 years (37 to 74) (**table I**). The mean BMI was 31.05 ± 1.57 (range 20.69 to 41.98). Five patients were healthy body weight or overweight (BMI < 30 kg/m^2) and eight patients were obese (BMI ≥ 30 kg/m^2). Individual patient details are shown in **appendix 1**.

Correlation analysis between tenocyte cell growth and gene expression of baseline tenocytes

There were no significant correlations between patient BMI and the tenocyte cell growth, as measured by alamarBlue assay,

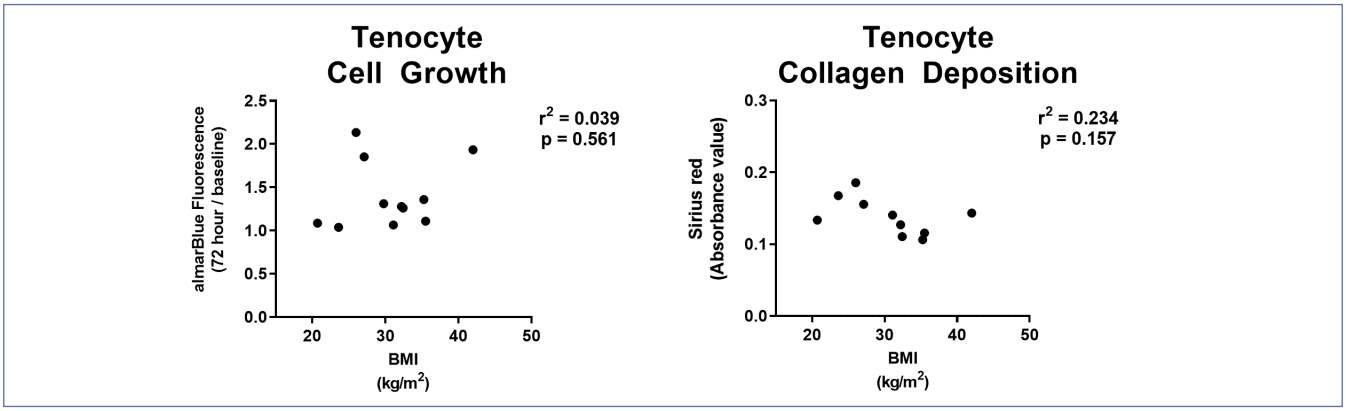

Figure 1. The correlation between BMI with [A] tenocyte cell growth and [B] tenocyte collagen deposition of baseline tenocytes over 72 hours.

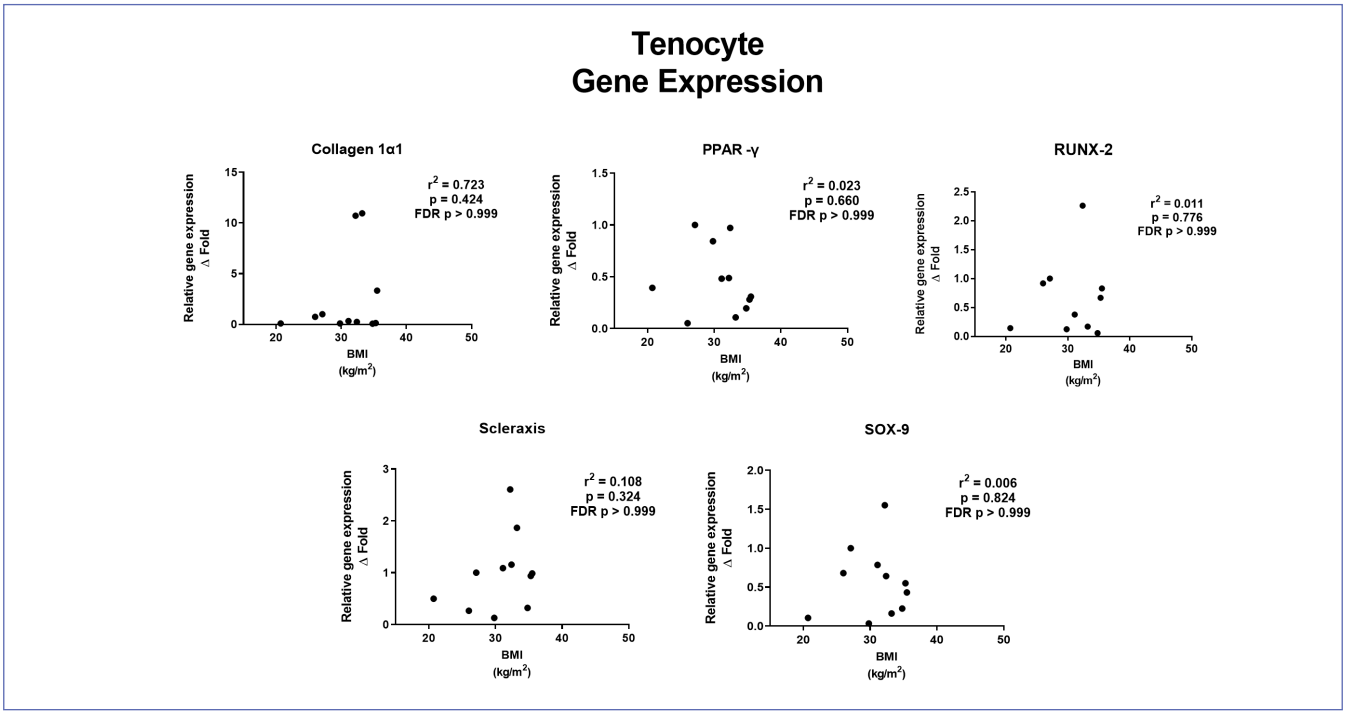

Figure 2. The correlation between BMI with tenocyte gene expression markers (collagen Iα1, *PPAR-γ*, *RUNX-2*, scleraxis and *SOX-9*).

Table I. Patient demographics, biomorphic measurements, and supraspinatus tear size.

Characteristic	Frequency
Sex	8 male; 5 female
Age	
Mean ± SEM	59.08 ± 2.80
Range	37 – 74
BMI (kg/m²)	
Mean ± SEM	31.05 ± 1.57

Characteristic	Frequency
Range	20.69 – 41.98
Ethnicity	
European	9
Māori	2
Asian	1
Pacific Peoples	1
Supraspinatus tear size	
Small	1
Medium	6
Large	5
Massive	1

Data are presented as mean ± SEM.

and collagen deposition, as measured by Sirius red assay, of the baseline tenocyte cells over 72 hours (**figure 1**). Correlation analyses did not demonstrate a significant correlation between patient BMI and baseline tenocyte gene expression markers (collagen Iα1, *PPAR-γ*, *RUNX-2*, scleraxis and *SOX-9*) (**figure 2**). In addition, there were no statistical differences between patients with BMI < 30 kg/m² and patients with BMI ≥ 30 kg/m² (**appendix 2**).

Correlation analysis between patient BMI and tenocyte cell growth, collagen deposition and gene expression in response to IGF-1, TGF-β and PDGF

Following 72 hours of treatment, tenocytes exposed to PDGF and TGF-β had significantly higher tenocyte growth, by 83.4% and 30.4% ($p < 0.05$), respectively. There was also higher collagen deposition with PDGF and TGF-β by 139.8% and 164.7% ($p < 0.05$), respectively (**appnedix 3**). However, IGF-1 did not affect cell growth or collagen deposition rate. There were no significant correlations between patient BMI and the tenocyte cell growth and collagen deposition in response to PDGF, TGF-β or IGF-1 (**figure 3**, **table II**). Furthermore, there was no statistical difference in tenocyte cell function in response to these tenogenic growth factors when comparing obese patients (BMI ≥ 30 kg/m²) with healthy and overweight patients (BMI < 30 kg/m²) (**appendix 4**).

PDGF treatment led to a 3-fold lower expression of the osteoblastic marker *ALP* and 4-fold higher expression of key adipose marker *PPAR-γ* ($p < 0.05$) compared to the untreated control. Expression of tenocyte-related mark-

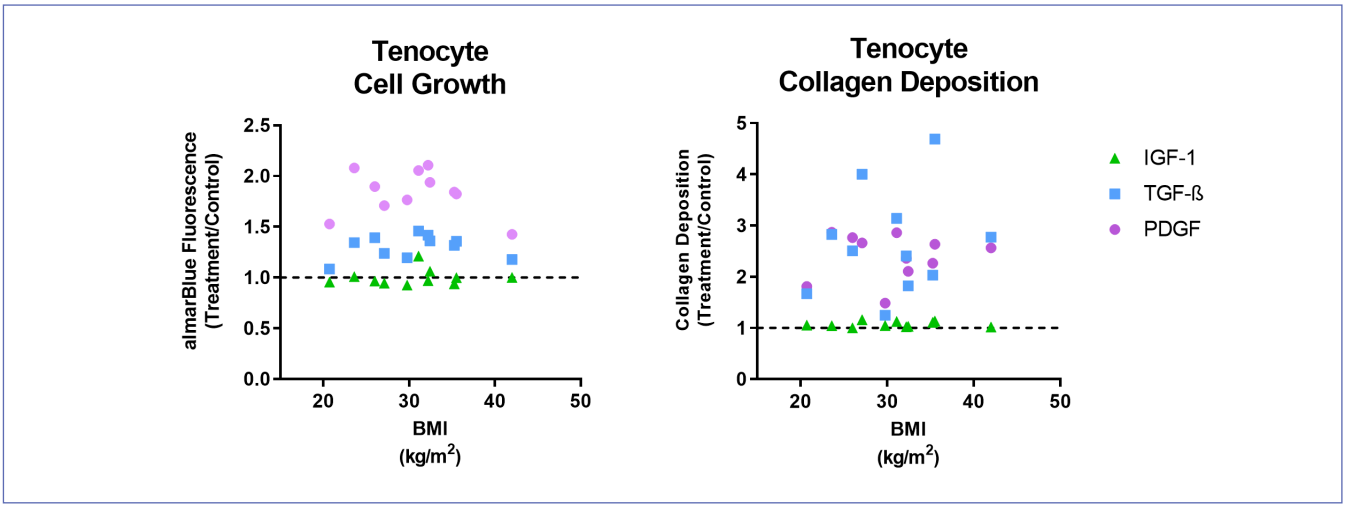

Figure 3. The correlation between BMI with tenocyte cell growth and tenocyte collagen deposition of tenocytes treated with IGF-1, TGF-β and PDGF tenocytes over 72 hours.

Table II. The correlation analyses between BMI with tenocyte cell growth and tenocyte collagen deposition of tenocytes in response to IGF-1, TGF-β and PDGF over 72 hours.

	R Squared	P-value
Tenocyte Cell Growth		
IGF-1	0.020	0.68
TGF-β	0.023	0.66
PDGF	0.034	0.59
Tenocyte Collagen Deposition		
IGF-1	0.002	0.91
TGF-β	0.041	0.55
PDGF	0.011	0.76

ers scleraxis, collagen Iα1 and chrondrocytic marker *SOX-9* were not significantly different. Tenocytes treated with TGF-β had 3-fold higher expression levels of collagen Iα, 50-fold higher expression of scleraxis, and 4-fold high expression of chondrocyte-related *SOX-9* compared to untreated cells ($p < 0.05$). There was also 5-fold lower expression levels of the osteoblast-related *ALP* ($p < 0.05$). IGF-1 treatment did not result in any significant changes in gene expression levels (**appendix 5**). Correlation analyses were also performed between patient BMI and tenocyte gene expression markers in response to each growth factor. No significant correlations were found after a FDR adjustment of P-values was performed (**figure 4**, **table III**). There were no statistical differences in gene expression between patients with BMI < 30 kg/m² and with BMI ≥ 30 kg/m² (**appendix 6**).

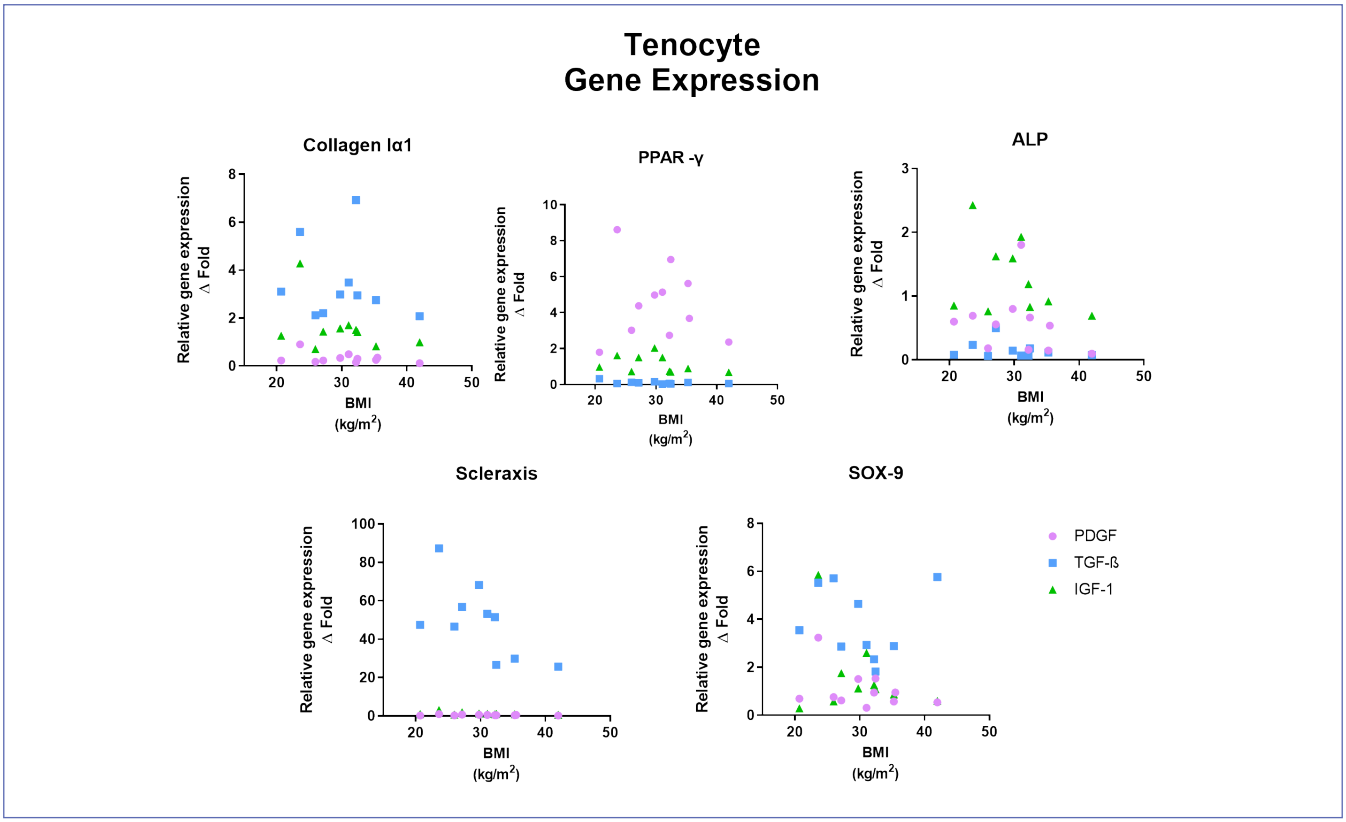

Figure 4. The correlation between BMI with gene expression markers (collagen Iα1, *PPAR-γ*, *ALP*, scleraxis and *SOX-9*) in response to IGF-1, TGF-β and PDGF over 72 hours.

Table III. The correlation analysis between BMI with gene expression markers (collagen Iα1, *PPAR-γ*, *ALP*, scleraxis and *SOX-9*) in response to IGF-1, TGF-β and PDGF over 72 hours.

Gene expression	R Squared	P-value	FDR-adjusted P-values
Collagen Iα1			
IGF-1	0.156	0.26	0.55
TGF-β	0.030	0.63	0.73
PDGF	0.141	0.26	0.55
PPAR-γ			
IGF-1	0.127	0.31	0.55
TGF-β	0.309	0.10	0.55
PDGF	0.018	0.70	0.75
ALP			
IGF-1	0.136	0.29	0.55
TGF-β	0.061	0.49	0.62
PDGF	0.057	0.48	0.62
SCX			
IGF-1	0.222	0.17	0.55
TGF-β	0.404	0.05	0.55
PDGF	0.105	0.33	0.55
SOX-9			
IGF-1	0.098	0.38	0.57
TGF-β	0.003	0.89	0.89
PDGF	0.126	0.28	0.55

DISCUSSION

Contrary to our hypothesis, here we have demonstrated that patient BMI has no direct effect on the characteristics of tenocytes derived from torn supraspinatus tendons, with no significant changes in cellular behaviour, gene expression or response to growth factor treatment. These findings have significant clinical implications, as it implies that growth factor treatment will be effective in patients regardless of their BMI.

Globally, there is an increasing trend in obesity prevalence that poses high healthcare costs, morbidity and societal burden (34). Overweight and obese individuals represent over a third of the worldwide population and are projected to make up over 60% by 2030 (35, 36). Recently a systematic review found that obese patients were at a 2-fold greater risk of having rotator cuff tears and 3-fold greater risk of re-tear after rotator cuff surgery (13).

The cellular mechanisms that underlie the relationship between obesity and rotator cuff tendinopathy are not well characterised (13). One explanation is that adipose tissue releases pro-inflammatory cytokines and hormones that influence activities in tenocytes, which directly modify the tendon healing response (37). Because many of these bioactive peptides secreted by adipose tissue increase in a near-linear fashion to body fat (38), we speculated that patient BMI may be a significant factor in altering tenocyte behaviour and gene expression. High circulating concentrations of adipokines may be acting as a prolonged disruptor of tendon healing in obese patients (14, 15, 25, 39). A recent animal study reported leptin levels, an adipokine, were negatively correlated with load to failure and worse histological structure of the repaired tendon-bone interface after rotator cuff surgery (40). However, in this current study, no significant correlation was found between patient BMI and tenocyte growth, collagen production or gene expression profile. Previous studies have demonstrated that tenocytes isolated from distinct tissue have a distinct cellular profile and decreased capacity to produce ECM components compared to healthy cells (41–43). They also respond differently than healthy cells to growth factor treatments, including TGF-β1 and BMP-2 (41, 44). We hypothesise that the disease stage-specific expression of fibrotic mediators may have a greater effect on cellular function than the comparatively minor effects of obesity, which may have masked any influence of BMI on tenocyte function and gene expression in this study. Further studies should investigate the effects of

BMI in tenocytes derived from healthy tendon and earlier stages of tendon disease.

Growth factors are an area of active research, and several different growth factors have shown success *in vitro* improving tendon healing including, IGF-1 (45), TGFβ (46, 47) and PDGF (48–50). Tendon healing involves the production and release of multiple growth factors at various phases of healing. The roles of specific growth factors typically work synergistically with other signalling molecules to increase cellularity and promote regeneration (21, 51, 52).

PDGF has been widely studied for improving tendon healing. Previous *in vitro* studies have yielded positive results with potent increases in tenocyte proliferation and collagen production (50,53). The effects of PDGF in animals models of tendon healing have been varied and inconsistent (48–50, 53, 54). In this present study, treatment of tenocytes with PDGF resulted in lower the expression of the osteoblastic marker *ALP* and higher expression of adipose marker *PPAR-γ*. Similar changes have also been reported in previous studies (31, 50). In diseased tendons, PDGF expression is increased and associated with hypercellularity (55). These findings suggest that PDGF may be promoting de-differentiation away from a tenocytic phenotype, and PDGF may be less suitable for improving tendon healing outcomes.

In previous *in vitro* studies, TGF-β has been demonstrated to promote the formation of fibrous tissue through the stimulation of collagen synthesis, regulation of matrix metalloproteinases and proliferation of fibroblasts (56–58). In this study, we found higher tenocyte growth and collagen deposition with TGF-β that was associated with higher expression of tenocyte-related genes, collagen Iα and scleraxis. These findings suggest that TGF-β may be tilting the balance of gene expression, favouring collagen and ECM synthesis. Interestingly, there was also a higher expression of chondrocyte-related *SOX-9*, suggesting TGF- β treatment may be favouring a chondrocytic response, which has been observed in previous studies (31, 59, 60).

IGF-1 did not influence tenocyte growth, collagen synthesis or gene expression. Previous *in vitro* and *in vivo* studies have shown IGF-1 increases cell proliferation and collagen synthesis in tendon (61–64). IGF-1 has structural similarities to insulin, allowing it to bind to insulin receptors and is involved in the anabolic response of tendons to loading (65). These differences between our research findings and those previously published could be the result of variations in culture conditions that can affect cell responses and that tenocytes were derived from different tendon types.

This study does have several limitations. Although we used clinically relevant samples harvested from supraspinatus tendons of patients undergoing rotator cuff surgery, there are inevitably confounding variables related to age, the severity of rotator cuff pathology and environmental exposures. In addition, these findings are limited in scope to only adult patients and patients were excluded if they had previous shoulder surgery; or had rheumatoid arthritis or other systemic inflammatory diseases.The sample size was also relatively small and did not allow the adjustment for these confounders, which may add to the impact of subjective variation. In this study, we also only had five participants with BMI under 30 kg/m^2 making it possible that we have underestimated the effect of obesity as most participants were obese (BMI ≥ 30 kg/m^2) (27).

CONCLUSIONS

This is the first study to investigate the effects of patient BMI on human tenocyte behaviour and response to growth factor treatment. In summary, we found no direct correlation between BMI and tenocyte growth, collagen synthesis or gene expression profile. These findings have significant clinical implications. They suggest that growth factor treatment for improving tendon-bone healing will be equally effective in non-obese and obese patients and can potentially overcome any deleterious effects occurring from obesity.

FUNDINGS

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DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

SMB, JTM, DSM: study conceptualization and design, data interpretation, article drafting. SMB, SK, YEP, DF, BC: data acquisition. ND: ethics required for the acquisition of data. All authors: revision of the manuscript and approval of the submitted manuscript.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

REFERENCES

1. Jain NB, Higgins LD, Losina E, Collins J, Blazar PE, Katz JN. Epidemiology of musculoskeletal upper extremity ambulatory surgery in the United States. BMC Musculoskelet Disord 2014;15(1):4.
2. Gomoll AH, Katz JN, Warner JJ, Millett PJ. Rotator cuff disorders: recognition and management among patients with shoulder pain. Arthritis Rheum 2004;50(12):3751-61.
3. Park HB, Gwark JY, Im JH, Jung J, Na JB, Yoon CH. Factors Associated with Atraumatic Posterosuperior Rotator Cuff Tears. J Bone Joint Surg Am 2018;100(16):1397-405.
4. Carr A, Cooper C, Campbell MK, *et al*. Effectiveness of open and arthroscopic rotator cuff repair (UKUFF) a randomised controlled trial. Bone Jt J 2017;99-B(1):107–15.
5. Hein J, Reilly JM, Chae J, Maerz T, Anderson K. Retear Rates After Arthroscopic Single-Row, Double-Row, and Suture Bridge Rotator Cuff Repair at a Minimum of 1 Year of Imaging Follow-up: A Systematic Review. Arthroscopy 2015;31(11):2274-81.
6. Galatz LM, Ball CM, Teefey SA, Middleton WD, Yamaguchi K. The Outcome and Repair Integrity of Completely Arthroscopically Repaired Large and Massive Rotator Cuff Tears. J Bone Jt Surg - Ser A 2004;86(2):219–24.
7. Iannotti JP, Deutsch A, Green A, *et al*. Time to failure after rotator cuff repair: A prospective imaging study. J Bone Jt Surg - Ser A 2013;95(11):965–71.
8. Miller BS, Downie BK, Kohen RB, *et al*. When do rotator cuff repairs fail? Serial ultrasound examination after arthroscopic repair of large and massive rotator cuff tears. Am J Sports Med 2011;39(10):2064–70. h
9. Gumina S, Candela V, Passaretti D, *et al*. The association between body fat and rotator cuff tear: the influence on rotator cuff tear sizes. J Shoulder Elb Surg 2014;23(11):1669–74.
10. Kim YK, Jung KH, Kim JW, Kim US, Hwang DH. Factors affecting rotator cuff integrity after arthroscopic repair for medium-sized or larger cuff tears: a retrospective cohort study. J Shoulder Elb Surg 2018;27(6):1012–20.
11. Oliva F, Piccirilli E, Berardi AC, Frizziero A, Tarantino U, Maffulli N. Hormones and tendinopathies: The current evidence. Br Med Bull 2016;117(1):39–58.
12. Oliva F, Osti L, Padulo J, Maffulli N. Epidemiology of the rotator cuff tears: a new incidence related to thyroid disease. Muscles Ligaments Tendons J 2014;4(3):309.
13. Macchi M, Spezia M, Elli S, Schiaffini G, Chisari E. Obesity increases the risk of tendinopathy, tendon tear and rupture, and postoperative complications: A systematic review of clinical studies. Clin Orthop Relat Res 2020;478(8):1839–47.
14. Abate M, Salini V, Andia I. How Obesity Affects Tendons? Adv Exp Med Biol 2016;920:167–77.
15. Franceschi F, Papalia R, Paciotti M, *et al*. Obesity as a risk factor for tendinopathy: a systematic review. Int J Endocrinol 2014;2014:670262.
16. Warrender WJ, Brown OL, Abboud JA. Outcomes of arthroscopic rotator cuff repairs in obese patients. J Shoulder Elb Surg 2011;20(6):961–7.
17. Ateschrang A, Eggensperger F, Ahrend MD, Schröter S, Stöckle U, Kraus TM, *et al*. Obesity causes poorer clinical results and higher re-tear rates in rotator cuff repair. Arch Orthop Trauma Surg 2018;138(6):835–42.
18. Zumstein MA, Lädermann A, Raniga S, Schär MO. The biology of rotator cuff healing. Orthop Traumatol Surg Res 2017;103(1S):S1–S10.
19. Leong NL, Kator JL, Clemens TL, James A, Enamoto-Iwamoto M, Jiang J. Tendon and Ligament Healing and Current Approaches to Tendon and Ligament Regeneration. J Orthop Res 2020 Jan38(1):7–12.
20. Via AG, De Cupis M, Spoliti M, Oliva F. Clinical and biological aspects of rotator cuff tears. Muscles Ligaments Tendons J 2013;3(2):70–9.
21. Würgler-Hauri CC, Dourte LM, Baradet TC, Williams GR, Soslowsky LJ. Temporal expression of 8 growth factors in tendon-to-bone healing in a rat supraspinatus model. J shoulder Elb Surg 2007;16(5 Suppl):S198–203.
22. Oliva F, Via AG, Maffulli N. Role of growth factors in rotator cuff healing. Sports Med Arthrosc Rev 2011;19(3):218–26.
23. Prabhath A, Vernekar VN, Sanchez E, Laurencin CT. Growth factor delivery strategies for rotator cuff repair and regeneration. Int J Pharm 2018;544(2):358–71.
24. Rechardt M, Viikari-Juntura E, Shiri R. Adipokines as predictors of recovery from upper extremity soft tissue disorders. Rheumatology (Oxford) 2014;53(12):2238–42.
25. Abate M, Schiavone C, Salini V, Andia I. Occurrence of tendon pathologies in metabolic disorders. Rheumatology (Oxford) 2013;52(4):599–608.
26. Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: an endocrine organ. Arch Med Sci 2013;9(2):191–200.
27. Gadde KM, Martin CK, Berthoud HR, Heymsfield SB. Obesity: Pathophysiology and Management. J Am Coll Cardiol 2018;71(1):69–84.
28. Dakin SG, Martinez FO, Yapp C, *et al*. Inflammation activation and resolution in human tendon disease. Sci Transl Med 2015;7(311):311ra173.
29. Matthews TJW, Hand GC, Rees JL, Athanasou NA, Carr AJ. Pathology of the torn rotator cuff tendon. Reduction in potential for repair as tear size increases. J Bone Jt Surg - Ser B 2006;88(4):489–95.
30. Chhana A, Callon KE, Dray M, *et al*. Interactions between tenocytes and monosodium urate monohydrate crystals: Implications for tendon involvement in gout. Ann Rheum Dis 2014;73(9):1737–41.
31. Musson DS, Tay ML, Chhana A, *et al*. Lactoferrin and parathyroid hormone are not harmful to primary tenocytes in vitro, but PDGF may be. Muscles Ligaments Tendons J 2017;7(2):215–22.
32. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 2001;25(4):402–8.
33. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J R Stat Soc Ser B 1995;57(1):289–300.
34. Inoue Y, Qin B, Poti J, Sokol R, Gordon-Larsen P. Epidemiology of Obesity in Adults: Latest Trends. Curr Obes Rep 2018;7(4):276-288.
35. Ng M, Fleming T, Robinson M, *et al*. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: A systematic analysis for the Global Burden of Disease Study 2013. Lancet 2014;384(9945):766–81.

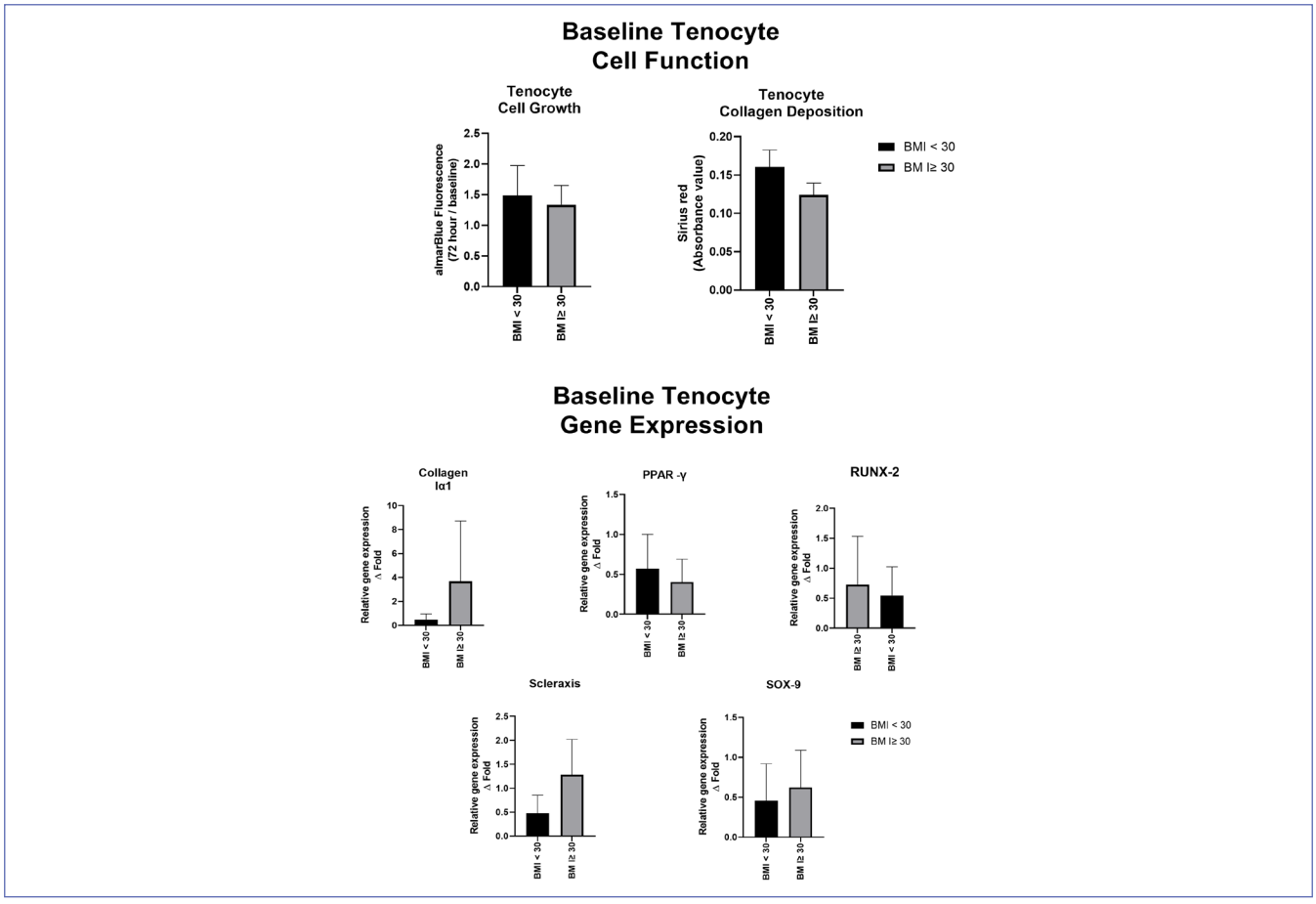
36. Stevens GA, Singh GM, Lu Y, *et al*. National, regional, and global trends in adult overweight and obesity prevalences. Popul Health Metr 2012;10(1):22.
37. Conde J, Gomez R, Bianco G, *et al*. Expanding the adipokine network in cartilage: Identification and regulation of novel factors in human and murine chondrocytes. Ann Rheum Dis 2011;70(3):551–9.
38. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395(6704):763–70.
39. Scott A, Zwerver J, Grewal N, *et al*. Lipids, adiposity and tendinopathy: is there a mechanistic link? Critical review. Br J Sports Med 2015;49(15):984–8.
40. Bolam SM, Park YE, Konar S, *et al*. Obesity Impairs Enthesis Healing After Rotator Cuff Repair in a Rat Model. Am J Sports Med 2021;49(14):3959–69.
41. Morita W, Snelling SJB, Wheway K, Watkins B, Appleton L, Murphy RJ, *et al*. Comparison of Cellular Responses to TGF-β1 and BMP-2 Between Healthy and Torn Tendons. Am J Sports Med 2021;49(7):1892–903.
42. Kendal AR, Layton T, Al-Mossawi H, *et al*. Multi-omic single cell analysis resolves novel stromal cell populations in healthy and diseased human tendon. Sci Rep 2020;10(1):13939.
43. Dakin SG, Ly L, Colas RA, *et al*. Increased 15-PGDH expression leads to dysregulated resolution responses in stromal cells from patients with chronic tendinopathy. Sci Rep 2017;7(1):11009.
44. Goodier HCJ, Carr AJ, Snelling SJB, *et al*. Comparison of transforming growth factor beta expression in healthy and diseased human tendon. Arthritis Res Ther 2016;18:48.
45. Dines JS, Grande DA, Dines DM. Tissue engineering and rotator cuff tendon healing. J Shoulder Elbow Surg 2007;16(5 Suppl):S204–7.
46. Arimura H, Shukunami C, Tokunaga T, *et al*. TGF-β1 Improves Biomechanical Strength by Extracellular Matrix Accumulation Without Increasing the Number of Tenogenic Lineage Cells in a Rat Rotator Cuff Repair Model. Am J Sports Med 2017;45(10):2394–404.
47. Kim HM, Galatz LM, Das R, Havlioglu N, Rothermich SY, Thomopoulos S. The role of transforming growth factor beta isoforms in tendon-to-bone healing. Connect Tissue Res 2011;52(2):87–98.
48. Tokunaga T, Ide J, Arimura H, *et al*. Local application of gelatin hydrogel sheets impregnated with platelet-derived growth factor BB promotes tendon-to-bone healing after rotator cuff repair in rats. Arthrosc - J Arthrosc Relat Surg 2015;31(8):1482–91.
49. Hee CK, Dines JS, Dines DM, *et al*. Augmentation of a rotator cuff suture repair using rhPDGF-BB and a type i bovine collagen matrix in an ovine model. Am J Sports Med 2011;39(8):1630–9.
50. Caliari SR, Harley BAC. Composite growth factor supplementation strategies to enhance tenocyte bioactivity in aligned collagen-GAG scaffolds. Tissue Eng - Part A 2013;19(9–10):1100–12.
51. Docheva D, Müller SA, Majewski M, Evans CH. Biologics for tendon repair. Adv Drug Deliv Rev 2015;84:222–39.
52. Kobayashi M, Itoi E, Minagawa H, *et al*. Expression of growth factors in the early phase of supraspinatus tendon healing in rabbits. J Shoulder Elb Surg 2006;15(3):371–7.
53. Thomopoulos S, Zaegel M, Das R, *et al*. PDGF-BB released in tendon repair using a novel delivery system promotes cell proliferation and collagen remodeling. J Orthop Res 2007;25(10):1358–68.
54. Kovacevic D, Gulotta LV, Ying L, Ehteshami JR, Deng X-HH, Rodeo SA. rhPDGF-BB Promotes Early Healing in a Rat Rotator Cuff Repair Model. 2015;473(5):1644–54.
55. Rolf CG, Fu BSC, Pau A, Wang W, Chan B. Increased cell proliferation and associated expression of PDGFRβ causing hypercellularity in patellar tendinosis. Rheumatology 2001;40(3):256–61.
56. Leask A, Abraham DJ. TGF-β signaling and the fibrotic response. FASEB J 2004;18(7):816–27.
57. Pohlers D, Brenmoehl J, Löffler I, *et al*. TGF-beta and fibrosis in different organs - molecular pathway imprints. Biochim Biophys Acta 2009;1792(8):746–56.
58. Pryce BA, Watson SS, Murchison ND, Staverosky JA, Dünker N, Schweitzer R. Recruitment and maintenance of tendon progenitors by TGFβ signaling are essential for tendon formation. Development 2009;136(8):1351–61.
59. Farhat YM, Al-Maliki AA, Chen T, *et al*. Gene Expression Analysis of the Pleiotropic Effects of TGF-β1 in an In Vitro Model of Flexor Tendon Healing. PLoS One 2012;7(12):e51411.
60. Holladay C, Abbah SA, O'Dowd C, Pandit A, Zeugolis DI. Preferential tendon stem cell response to growth factor supplementation. J Tissue Eng Regen Med 2016;10(9):783–98.
61. Olesen JL, Heinemeier KM, Gemmer C, Kjær M, Flyvbjerg A, Langberg H. Exercise-dependent IGF-I, IGFBPs, and type I collagen changes in human peritendinous connective tissue determined by microdialysis. J Appl Physiol 2007;102(1):214–20.
62. Hansen M, Boesen A, Holm L, Flyvbjerg A, Langberg H, Kjaer M. Local administration of insulin-like growth factor-I (IGF-I) stimulates tendon collagen synthesis in humans. Scand J Med Sci Sport 2013;23(5):614–9.
63. Herchenhan A, Bayer ML, Eliasson P, Magnusson SP, Kjaer M. Insulin-like growth factor I enhances collagen synthesis in engineered human tendon tissue. Growth Horm IGF Res 2015;25(1):13–9.
64. Wieser K, Farshad M, Meyer DC, Conze P, von Rechenberg B, Gerber C. Tendon response to pharmaco-mechanical stimulation of the chronically retracted rotator cuff in sheep. Knee Surgery, Sport Traumatol Arthrosc 2015;23(2):577–84.
65. Doessing S, Heinemeier KM, Holm L, *et al*. Growth hormone stimulates the collagen synthesis in human tendon and skeletal muscle without affecting myofibrillar protein synthesis. J Physiol 2010;588(2):341–51.

SUPPLEMENTS

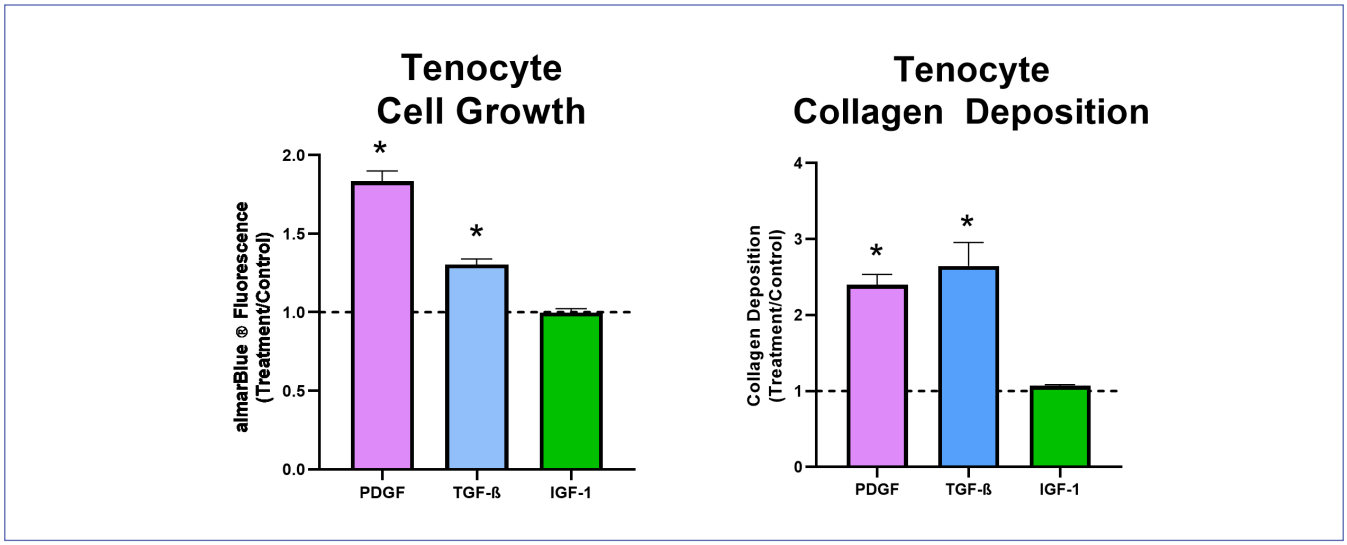
Appendix 1. Individual patient demographics, biomorphic measurements, and supraspinatus tear size.

Gender	Age	BMI (kg/m²)	Ethnicity	Tear size
Female	73	20.7	European	Medium
Female	64	23.6	Asian	Medium
Male	63	26	European	Medium
Female	74	27.1	European	Small
Male	54	29.7	Māori	Medium
Male	63	31.1	European	Medium
Male	37	32.2	Pacific Island	Small
Male	64	32.4	European	Medium
Female	55	33.2	European	Large
Male	46	34.8	European	Large
Female	61	35.3	European	Large
Male	60	35.5	European	Large
Male	54	42.0	Māori	Massive

Appendix 2. Comparison in baseline tenocyte cell function and gene expression between patients with BMI under 30 and BMI over 30.

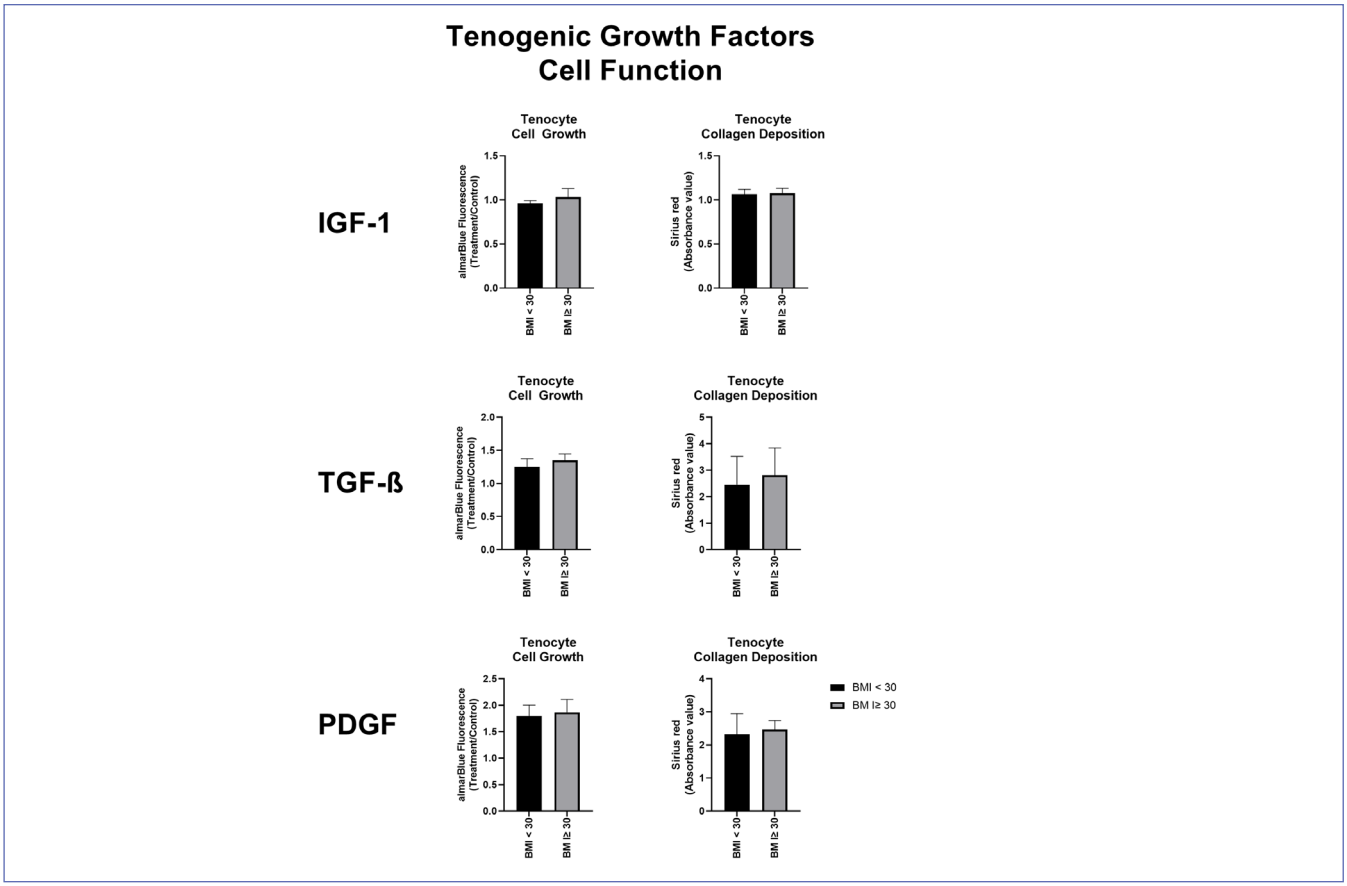


Appendix 3. Effects of the growth factors PDGF, TGF-β on IGF-1 tenocyte cell growth, determined by alamarBlue assay, and tenocyte collagen production, determined by Sirius red assay.

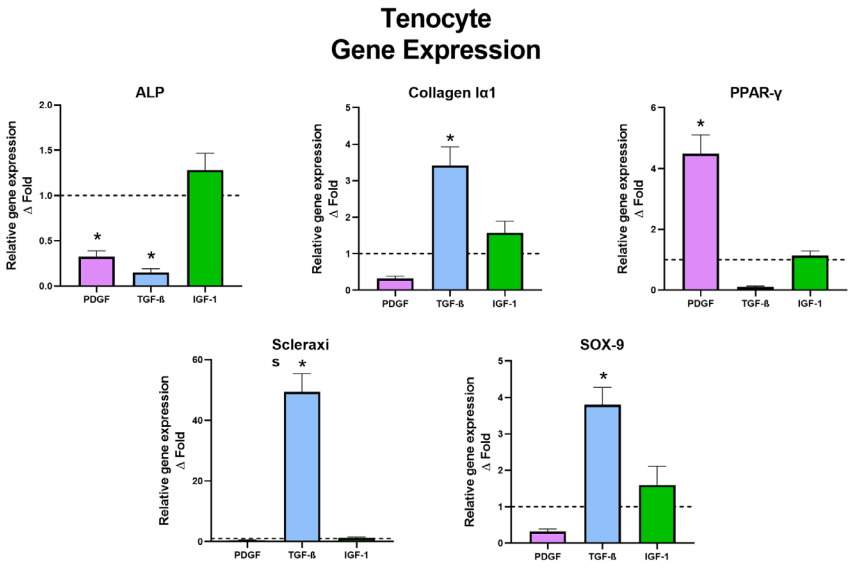


Data are presented as the mean ratio of control ± SEM. *: significantly different from control ($p < 0.05$).

Appendix 4. Comparison in tenocyte cell function in response to TGF-β on IGF-1 tenocyte cell growth and collagen production between patients with BMI under 30 and BMI over 30.



Appendix 5. Effects of growth factors PDGF, TGF-β and IGF-1 on tenocyte gene expression.



Data are presented as means ± SEM. *: significantly different from control ($p < 0.05$).

Appendix 6. Comparison in gene expression in response to TGF-β on IGF-1 tenocyte cell growth and collagen production between patients with BMI under 30 and BMI over 30.