1. Introduction

Peripheral artery disease (PAD) is characterized by occlusion of the arteries of the lower extremities [1, 2]. Femoral, popliteal, tibial, and peroneal arteries are most commonly affected, leading to a higher risk of lower-extremity amputation [3, 4].

The risk of developing PAD is four times higher in the presence of diabetes mellitus than in its absence. Diabetes is rated as the strongest risk factor for the development of PAD [7-11], with a one percentage rise in HbA1c increasing the risk of developing PAD by 26% [5, 6]. Studies investigating the kinematics of people with PAD and type 2 diabetes mellitus (T2D) as separate morbidities report distinct gait variability, particularly in the ankle joint and knee joint [12].

Patients living with T2D alone tend to exhibit reduced ankle joint dorsiflexion and knee flexion during gait [13-16], particularly in the presence of peripheral neuropathy [17-18]. Apart from T2D, peripheral arterial disease alone was found to affect the ankle joint by increasing dorsiflexion during gait and to reduce knee joint flexion [19-22]. To date, there have been no studies investigating the effect of both PAD and T2D as comorbidities on ankle joint dorsiflexion and knee flexion.

Altered biomechanical conditions brought about by both PAD and T2D may put this patient population at higher risk of developing deformities of the foot structure and muscle weakness, which in turn may result in higher predisposition to tissue stress [23], ulceration [24-26], falls, and even amputations [27]. Studies by Formosa et al. highlight the importance of meticulous and in-depth
biomechanical examination with particular focus on joint movement and foot deformities [28, 29].

Many studies concentrated on the effect of diabetes on lower limb kinematics, but the impact of PAD is frequently disregarded [13-18]. It is plausible that the previously mentioned alteration in lower limb kinematics is secondary to nerve damage and muscle weakness resulting from long-standing ischemia. Muscle function is greatly dependent on vascular supply and the lack of it may alter the gait.

3D gait analysis may provide new insight into the mechanisms underlying altered biomechanics that may cause an increased risk of foot ulceration in patients with T2D and PAD. Thus, the purpose of this study was to investigate the impact of PAD as a complication of T2D on ankle joint dorsiflexion and knee joint flexion angles utilizing an optoelectronic motion analysis system.

2. Methods

Ethical approval was granted by the University Research Ethics Committee and all consenting participants were treated according to the Declaration of Helsinki [30]. Using a purposive sampling technique, 90 participants (180 limbs) aged 50-75 years, presenting with T2D and PAD of varying severity, were recruited from local health centers. Participants with the following conditions were excluded from this study to avoid influences other than PAD on limb complications [12, 20, 22, 31]:

- History of musculoskeletal pathologies such as rheumatoid arthritis (RA), osteoarthritis (OA), and foot deformities
- History of amputation
- Neuromuscular disorders especially Charcot-Marie-Tooth disease
- Peripheral neuropathy

Participants were also excluded if they required walking aids such as crutches, wheelchairs, or prosthesis [12, 20, 22, 31].

In order to ensure the validity, precision, reliability, and integrity of the research study, thorough selection of participants that matched the inclusion criteria was carried out and a representative sample of the general population was recruited. Rigor and prevention of bias were applied throughout the study.

The tools selected to conduct this study, including the neurothesiometer, neuropen, ankle brachial pressure index (ABPI), toe brachial pressure index (TBPI), force plate, and 3D motion capture system, were all certified as reliable and valid and were deemed to be a gold standard tool for the diagnosis of neuropathy, level of PAD, and measurement of the kinetics and kinematics of the human body, respectively.

Prior to recruitment, the participants were screened using a 10g monofilament and a neurothesiometer to exclude the presence of peripheral neuropathy [12, 32, 33]. Presence and severity of PAD were assessed using TBPI, which compares the systolic pressure of the brachial artery with the pressure of the big toe, as per standard protocol [34]. Prior to using neurothesiometer, neuropen, ABPI, and TBPI, the participants were given enough time to acclimatize to the environment and rest for not less than ten minutes.

The participants recruited were categorized into three groups according to their TBPI results:

- Participants with a TBPI greater than 0.7 were classified as T2D without PAD (control group, n = 30).
- Participants with a TBPI between 0.64-0.7 were classified as patients with mild PAD (T2D + mild PAD group, n = 32).
- Participants scoring a TBPI of less than 0.64 were categorized as patients with severe PAD (T2D + severe PAD group, n = 28).

Retroreflective markers were placed on specific anatomical landmarks as instructed by the Plug-In Gait model (Vicon, OMG, Oxford, UK) [35]. Prior to data collection, a static subject calibration was performed to customize a subject skeleton, to define the relationship between each marker, and to permit real-time conversion of images coordinated for each marker versus virtual coordinates captured by each camera [36]. Each participant was asked to walk at a self-selected speed along a 10 meter walkway during which ten infrared cameras captured his motion in the calibrated volume at a rate of 100 Hz. Ten cameras were set up along the laboratory walls to avoid unwanted movement or damage during the procedure, while maintaining enough flexibility to adjust for the position required and avoid ‘dead space’, i.e. any space that does not lie within the field of view of the cameras. The walking trials deemed to be incorrect were discarded, as per standard gait analysis practice. One session (recording) for each participant comprised a total number of 24 trials (walks), which were then all averaged in preparation for statistical analysis and interpretation.

In this study, reliability was ensured by using the same equipment throughout the study to ensure consistency in the results. Thorough attention was also given during data collection to ensure high quality data by paying attention to hardware set-up such as camera settings, capture volume, position and number of cameras, calibration techniques, and marker placement. The system resolution is affected by the capture volume setting, and was thus regulated to compromise between

Abbreviations:

| Abbreviation | Description |
|--------------|-------------|
| ABPI         | ankle brachial pressure index |
| ANOVA        | analysis of variance |
| EMG          | electromyograph |
| HSD          | honest significant difference |
| OA           | osteoarthritis |
| RA           | rheumatoid arthritis |
| PAD          | peripheral artery disease |
| T2D          | type 2 diabetes mellitus |
| TBPI         | toe brachial pressure index |
| SPSS         | Statistical Package of the Social Services |
the activity being recorded and the quality resolution of the system.

After the data captured had been processed using Butterworth filtering, maximum angular movement of the ankle joint and that of the knee joint (sagittal plane movement) were recorded, from which mean maximum ankle dorsiflexion angles and knee flexion angles were derived.

The Kolmogorov-Smirnov test was used to verify the distribution of the data. The results were analyzed statistically using one-way ANOVA, while a Tukey’s honest significant difference (HSD) test (as a post-hoc test) was also applied to determine which group exhibited significant differences in the dorsiflexion and flexion angles [37]. All statistical analyses were conducted with the Statistical Package for the Social Services (SPSS, IBM) version [24].

3. Results

One-way ANOVA showed a statistically significant difference between the maximum ankle dorsiflexion angle of the control group (T2D only) and participants with both T2D and PAD (p = 0.001, Table 1). A statistically significant difference was also found between the maximum knee flexion angle of the control group and that of the participants with both T2D and PAD (p = 0.001, Table 2).

### Table 1. One-way ANOVA statistical results for maximum dorsiflexion at the ankle joint

| Group    | n  | Mean angles (degrees) | Std. deviation | p-value |
|----------|----|-----------------------|----------------|---------|
| Normal   | 60 | 17.22                 | 5.40           | 0.001*  |
| Mild     | 64 | 21.18                 | 4.96           |         |
| Severe   | 56 | 21.25                 | 5.36           |         |
| Total    | 180| 19.46                 | 5.65           | <0.001  |

### Table 2. One-way ANOVA statistical results for the maximum flexion at the knee joint

| Group    | n  | Mean angles (degrees) | Std. deviation | p-value |
|----------|----|-----------------------|----------------|---------|
| Normal   | 60 | 45.13                 | 9.91           | 0.001*  |
| Mild     | 64 | 29.67                 | 6.80           |         |
| Severe   | 56 | 27.38                 | 7.19           |         |
| Total    | 180| 34.27                 | 11.34          | 0.001*  |

Post-hoc analysis, for both the ankle joint and knee joint, concluded that there were differences in mean scores between both the control versus severe PAD group (difference of 4.04° for the ankle joint dorsiflexion and 17.75° for the knee joint flexion; p = 0.001) and between the control versus mild PAD group (difference of 3.96° for the ankle joint dorsiflexion and 15.46° for the knee joint flexion; p = 0.001, Tables 3 and 4). Both mild and severe PAD groups exhibited an increase in mean angles for both ankle joint and knee during maximum dorsiflexion/flexion.

### Table 3. Post-hoc statistical results for maximum dorsiflexion at the ankle joint

| Groups               | Mean difference (degrees) | p-value |
|----------------------|---------------------------|---------|
| Normal vs. severe    | 4.04                      | 0.001*  |
| Normal vs. mild      | 3.96                      | 0.007*  |
| Severe vs. mild      | 0.08                      | 0.998   |

### Table 4. Post-hoc statistical results for maximum flexion at the knee joint

| Groups               | Mean difference (degrees) | p-value |
|----------------------|---------------------------|---------|
| Normal vs. severe    | 17.75                     | 0.001*  |
| Normal vs. mild      | 15.46                     | 0.001*  |
| Severe vs. mild      | 0.29                      | 0.304   |

4. Discussion

This study is the first of the few existing studies [12, 20, 22] to explore the effects of PAD on lower limbs in a population with PAD as a complication of diabetes mellitus. It is also the first to investigate the effects of speed variation on the ankle joint and knee joint of patients with both T2D and PAD.

The results from this study confirm that patients with PAD as a comorbidity of T2D exhibited a greater mean maximum dorsiflexion of the ankle joint and reduced mean maximum flexion angles of the knee joint during the stance phase of gait than those with T2D only.

Previous studies have shown that patients with T2D only exhibit reduced mean maximum dorsiflexion of the ankle joint and reduced mean maximum flexion angles of the knee joint during the stance phase of gait [13, 16]. Although the control group in the present study achieved low angular measurements of long-standing diabetes mellitus, especially if uncontrolled, is known to be a deteriorative factor on nerves which in turn affect muscle function and gait. Studies investigating kinematics in people with diabetes only observed that these patients exhibited a reduction in ankle joint dorsiflexion and knee flexion during gait [13, 16], particularly in the presence of peripheral neuropathy [17, 18]. Although the control group in the present study achieved low angular measurements of
ankle joint dorsiflexion compared to normative expected values, the angular results were slightly higher when compared with those of diabetic participants in other studies. In our study, participants with good glycemic control acted as controls, which emphasizes the importance of well controlled blood glucose levels. It also highlights the influence of glycemic control on the kinematics of the lower limb.

The present study has recruited the largest sample size (n = 90) among those studies investigating the biomechanical alterations in T2D and/or PAD and assessed bilateral limbs (180 limbs in total). The participants in the study group had both T2D and PAD, and were compared with people with controlled T2D alone who acted as controls. To the best of our knowledge, this scenario has never been investigated before.

Even though both groups may have experienced glycosylation of the muscles because of the presence of diabetes, participants with PAD are also likely to exhibit compensation secondary to muscular weakness. As a compensatory mechanism, a reduction in the range of motion of one joint (knee joint) brings about an increase (overcompensation) in range of motion in another joint (ankle joint) with the purpose of maintaining an efficient forward movement [41]. This distinct difference between the effects of the two diseases may be attributed to an adaptation to a biomechanical function, or to a neuromuscular weakness brought about by peripheral arterial disease. Nerve damage and muscle weakness of the anterior, lateral, and posterior compartment of the ankle joint are hypothesized to limit dorsiflexion during gait [42, 43]. Moreover, alterations in lower limb biomechanics may cause an increase in tissue stress, with a consequent increase of tissue breakdown, especially among people with diabetes and PAD [23].

Being the farthest anatomical structure of the body, the calf muscles are more likely to be affected by glycrosylation and stiffness caused by prolonged ischemia and diabetes [38, 44]. This may result in an imbalance between the anterior and posterior compartment of the lower limb, causing the foot dorsiflexors to overwork and compensate for the deficient function of the plantar flexors.

Associated deformities of foot structure may cause high risk of foot ulceration in weight-bearing areas [45]. Thus, people with diabetes are at a higher risk of lower-limb amputations than healthy people, especially in the presence of PAD, which accelerates the risk by directly damaging nerves, blood vessels, and collagen synthesis [27].

5. Conclusions

People with PAD and T2D were known to have a greater ankle joint dorsiflexion and a reduced knee flexion compared to people living with T2D only. This difference between the two groups may be attributed to a compensatory mechanism during gait. Furthermore, the reduction in ankle joint dorsiflexion presented by the patients with T2D may be primarily the result of early stage neuropathy and not of reduced blood perfusion.

Participants suffering from severe PAD, with a TBPI score of less than 0.64, were found to have a 4.04 degree increase in the ankle joint during maximum dorsiflexion and a 17.75 degree reduced knee flexion compared to people with T2D only. This alteration may be attributed to a mechanism compensating for glycosylation and oxygen deprivation (i.e. ischemia) to the farthest anatomical structure of the body, the calf muscle.

The novel aspect of this study is the fact that the observations made question whether the expected decrease in the ankle joint dorsiflexion, commonly revealed in diabetes, may be secondary to early stage neuropathy and not secondary to reduced blood perfusion alone. Furthermore, the study highlights the importance of having well controlled blood glucose levels which impact the kinematics of the body during gait.

Changes in the biomechanical function of the lower limb, especially in high-risk patients, are known to cause an increase in tissue stress and tissue breakdown, leading to possible amputations and early mortality rates. This study proposes a new perspective for examining the high-risk foot and for better understanding the effects of PAD and diabetes on the lower limb.

6. Recommendations

A reduction in knee joint flexion angle was found to increase the risk of falling among participants with PAD [26], which should be investigated in future studies with special focus on patients with PAD as a complication of diabetes mellitus. It is also recommended to explore in more detail the ankle joint regarding neurological and mechanical effects on the angle of this joint in participants with diabetes mellitus and PAD to find out why there may be an increase in joint angle, as shown in this study.

Future studies may reinvestigate in more detail the effect on kinetics and kinematics of a population with combined T2D and PAD at different walking speeds, with the aim of improving the alterations in diabetes-related biomechanics.

Timing of maximum dorsiflexion/flexion and plantarflexion/extension of joints in relation to the phases of the gait cycle would be an interesting and valuable aspect to explore as little research is currently available in relation to this patient population. Such insight would help the clinician to understand better the biomechanical limitations of people with both conditions and to optimize treatments that improve mobility and prognosis in these individuals.

Future studies are also recommended to explore the effect of PAD in the presence of diabetes mellitus on the muscle power and timing of contraction during gait using surface EMG. Lack of vascularization to the muscle is considered to cause a possible reduction in muscle effectiveness which in turn may result in alterations in the range of motion of the joints [20].

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References

1. Ouriel K. Peripheral arterial disease. Lancer 2001. 358 :1257-1264.
2. Monteiro DP, Britto RR, Ribeiro Lages AC, Lopes Basílio M, de Oliveira Pires MC, Vieira Carvalho ML, Procopio RJ, Aparecida Gomes Pereira D. Heel-raise test in the assessment of individuals with peripheral arterial occlusive disease. Vasc Health Risk Manag 2013. 9:29-35.
3. Criqui MH. Peripheral arterial disease: epidemiological aspects. Vasc Med 2001. 6(1):3-7.
4. Centers for Disease Control and Prevention (CDC). Peripheral arterial disease (PAD) fact sheet. Retrieved December 8, 2017, from https://www.cdc.gov/dhdsp/data_statistics/fact_sheets/fs_pad.htm.
5. Jude EB, SO Oyibo, N Chalmers, AJ Boulton. Peripheral arterial disease in diabetic and nondiabetic patients: a comparison of severity and outcome. Diabetes Care 2001. 24(8):1433-1437.
6. Selvin E, Erlinger TP. Prevalence of and risk factors for peripheral arterial disease in the United States: Results from the national health and nutrition examination survey. Circulation 2004. 110(6):738-743.
7. Gili M, Orsello A, Gallo S, Felice Brizzi M. Diabetes-associated macrovascular complications: cell-based therapy a new tool? Endocrine 2013. 44(3):545-557.
8. Pitocco D, Tesauro M, Alessandro R, Ghirlanda G, Cardillo C. Oxidative stress in diabetes: implications for vascular and other complications. Int J Mol Sci 2013. 14(11):21525-21550.
9. Ogren M, Hedblad B, Engström G, Janzon L. Prevalence and prognostic significance of asymptomatic peripheral arterial disease in 68-year-old men with diabetes. Results from the population study ‘Men Born in 1914’ from Malmö, Sweden. Eur J Vasc Endovasc Surg 2005. 29(2):182-189.
10. Togliatto G, Trombetta A, Dentelli P, Gallo S, Rosso A, Cotogni P, Granata R, Falcioni R, Delale T, Ghigo E, Felice Brizzi M. Unacylted ghrelin induces oxidative stress resistance in a glucose tolerant diabetic foot model. Diabetes Care 2013. 36(1):137-1382.
11. Ellul C, Gatt, A. Transcutaneous calf-muscle electro-stimulation: A prospective treatment for diabetic claudicants? Diab Vasc Dis Res 2016. 13(6):442-444.
12. Myers SA, Johanning JM, Pipinos II, Schmid KK, Stergiou N. Vascular occlusion affects gait variability patterns of healthy younger and older individuals. Ann Biomed Eng 2013. 41(8):1692-1702.
13. Yavuzer G, I Yetkin, FB Toruner, N Koca, N Bolukbasi. Gait deviations of patients with diabetes with diabetes mellitus: looking beyond peripheral neuropathy. Eur J Med Phys 2006. 42(2):127-133.
14. Giacomozzi C, D’Ambrogi E, Stefano Cesinaro S, Macellari V, Uccioli L. Muscle performance and ankle joint mobility in long-term patients with diabetes. BMC Musculoskelet Disord 2008. 9:99.
15. Liu MW, Hsu WC, Lu TW, Chen HL., Liu HC. Patients with type II diabetes mellitus display reduced toe-obstacle clearance with altered gait patterns during obstacle-crossing. Gait Posture 2010. 31(1):93-99.
16. Ko SU, Stenholm S, Chia CW, Simonsick EM, Ferrucci L. Gait patterns alterations in older adults associated with type 2 diabetes in the absence of peripheral neuropathy - results from the Baltimore longitudinal study of aging. Gait Posture 2011. 34(4):548-552.
17. Worbel JS, Birkmeyer NJ, Dercoli JL., Connolly JE. Do clinical examination variables predict high plantar pressures in the diabetic foot? J Am Podiatr Med Assoc 2004. 93(5):367-372.
18. Zimny S, Schatz H, Pfohl M. The role of limited joint mobility in diabetic patients with an at-risk foot. Diabetes Care 2004. 27(4):942-946.
19. Crowther RG, Spinks WL, Leight AS, Quigley F, Golley J. Relationship between temporal-spatial parameters, gait kinematics, walking performance, exercise capacity, and physical activity level in peripheral arterial disease. J Vasc Surg 2007. 45(6):1172-1178.
20. Celis R, Pipinos II, Scott-Pandor MM, Myers SA, Stergiou N, Johanning JM. Peripheral arterial disease affects kinematics during walking. J Vasc Surg 2009. 49(1):127-132.
21. Koutakis P, Pipinos II, Myers SA, Stergiou N, Lynch TG, Johanning JM. Joint torques and powers are reduced during ambulation for both limbs in patients with unilateral claudication. J Vasc Surg 2010. 51(1):80-88.
22. Myers SA. Gait variability of peripheral arterial disease patients is similar before and after onset of claudication pain. J Clin Biomech 2011. 26(7):729-731.
23. Rao S, Saltzman HJ. Yack segmental foot mobility in individuals with and without diabetes and neuropathy. Clin Biomech (Bristol, Avon) 2007. 22(4):464-471.
24. Scott-Okafor HR, KK Silver, J Parker, T Almy-Albert, AW Gardner. Lower extremity strength deficits in peripheral arterial occlusive disease patients with intermittent claudication. Angiology 2001. 52:7-14.
25. Gardner AW, Montgomery PS. The relationship between history of falling and physical function in subjects with peripheral arterial disease. Vasc Med 2001. 6:223-227.
26. Bailey MC, Griffin KJ, Scott DJ. Clinical assessment of patients with peripheral arterial disease. Semin Intermed Radiol 2014. 31(4):292-299.
27. Weledji EP, Fokam P. Treatment of the diabetic foot - to amputate or not? BMC Surg 2014. 14:83.
28. Formosa C, Gatt A, Cheokalingam N. A critical evaluation of existing diabetic foot screening guidelines. Rev Diabet Stud 2016. 13(2-3):158-186.
29. Formosa C, Gatt A, Cheokalingam N. The importance of clinical biomechanical assessment of foot deformity and joint mobility in people living with type-2 diabetes within a primary care setting. Prim Care Diabetes 2013. 7(1):45-50.
30. World Medical Association. Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. JAMA 2013. 310(20):2191-2194.
31. King S, Vanicek N, Mockford KA, Coughlin PA. The effect of a 3-month supervised exercise programme on gait parameters of patients with peripheral arterial disease and intermittent claudication. Clin Biomech (Bristol Avon) 2012. 27(8):845-851.
32. Yates, B. Merriman’s assessment of the lower limb. Edinburgh, Churchill Livingstone, 2012.
33. Boulton AJ, Armstrong DG, Albert SF, Frykberg RG, Hellman R, Kirkman MS, Lavery LA, LeMaster JW, Mills JL Sr, Mueller MJ, Sheehan P, Wukich DK. Comprehensive foot examination and risk assessment: A report of the task force of the foot care interest group of the American diabetes association, with endorsement by the American Association of Clinical Endocrinologists. Phys Ther 2008. 88(11):1436-1443.
34. Gerhard-Herman MD, Gornik HL, Barrett C, Barsnes NR, Corriere MA, Drachman DE, Fleisher LA, Fowkes FG, Hamburg NM, Kinlay S, Lookstein R. AHA/ACC guidelines on the management of lower extremity peripheral arterial disease: A Report of the American College of Cardiology/American Heart Association task force on clinical practice guidelines. Circulation 2017. 135(12):e726-e779.
35. Vicon. Vicon Plug-in-Gait modelling instructions: Vicon Manual, Vicon Motion Systems. Oxford Metrics Ltd., Oxford, UK, 2002.
36. Sigal L. Marker base: motion capture, 2012. Retrieved December 8. 2015, from: http://www.cs.cmu.edu/~yaser/Lecture-3-MarkerBasedMocap.pdf.
37. Jackson SL. Statistics plain and simple. California, USA, Wadsworth, 2014.
38. Huisinga JM, Pipinos II, Johanning JM, Stergiou N. The effect of pharmacological treatment on gait biomechanics in peripheral arterial disease patients. J Neuroeng Rehabil 2010. 7:25.

39. Chen SJ, Pipinos II, Johanning J, Radovic M, Huisinga JM, Myers SA, Stergiou N. Bilateral claudication results in alteration in the gait biomechanics at hips and ankle joints. J Biomech 2008. 41(11):2506-2514.

40. Maugari AG, Archidiacono G. Efficient determination of sampling rate and sample size. Univ J Industr Busin Manag 2014. 2(4):103-110.

41. Sawacha Z, Gabriella G, Cristoferi G, Guiotto A, Avogaro A, Cobelli C. Diabetic gait and posture abnormalities: a biomechanical investigation through three-dimensional gait analysis. Clin Biomech (Bristol, Avon) 2010. 24(9):722-728.

42. Kuo AD. The six determinants of gait and the introverted pendulum analogy: A dynamic walking perspective. Hum Mov Sci 2007. 26:637-56.

43. Pipinos II, Judge AR, Sebby JT, Zhu Z, Swanson SA, Nella AA, Dodd SL. The myopathy of peripheral arterial occlusive disease: Part 2. Oxidative stress, neuropathy, and shift in muscle fibre type. Vasc Endovasc Surg 2008. 42(2):101-112.

44. Graf A, Judge JO, Ounpuu S, Thelen DG. The effect of walking speed on lower-extremity joint powers among elderly adults who exhibit low physical performance. Arch Phys Med Rehabil 2005. 86(11):2177-2183.

45. Ledoux WR, Shofer JB, Smith DG, Sullivan K, Hayes SG, Assal M, Reiber GE. Relationship between foot type, foot deformity, and ulcer occurrence in the high-risk diabetic foot. J Rehabil Res Dev 2005. 42(5):665-672.