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Non-invasive assessment of endothelial function in children and adolescents with type 1 diabetes mellitus using handgrip-MRI: A feasibility study

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Non-invasive assessment of endothelial function in children and adolescents with type 1 diabetes mellitus using handgrip-MRI: A feasibility study.

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Abstract

Background

Type 1 diabetes mellitus (T1D) in children and adolescents is associated with significant cardiovascular morbidity and mortality. Early detection of vascular dysfunction is key to management yet assessment is invasive – which is particularly challenging when considering pediatric patient populations. Recently, a novel approach using isometric handgrip exercise (IHE) during magnetic resonance imaging (MRI) has been developed to evaluate coronary endothelial function in adults.

Aims

This project aimed to assess coronary vessel response to IHE using MRI in children with T1D children and healthy controls. In addition, we compared the groups in terms of surrogate markers of intima media alteration and arterial stiffness, i.e. carotid intima media thickness (CIMT) and aortic pulse wave velocity (PWV).

Methodology

Community-dwelling healthy volunteers (<18 years-old) and children with type 1 diabetes mellitus (disease duration ≥ 5 years) were recruited. IHE-MRI studies were conducted and measurements were recorded at rest (baseline) and under stress (IHE at 30% maximal effort). Carotic Ultrasound and Sphygmocor CPV System were used to assess CIMT and PWV respectively. Student's T-tests were used to compare results between groups.

Results

Seven children with T1D (3 female, 4 male, mean age in decimals 14.8 ± 1.9 years) and 16 healthy children (7 female, 9 male, mean age in decimals 14.2 ± 2.4 years) as control. IHE-MRI showed a mean area change of 18.84% ± 10.72% (from mean 5.4 mm^2 at rest to mean 6.39 mm^2 under stress, p=0.0004) in control participants and a mean area change of 10.5% ± 28.05% (from mean 7.17 mm^2 at rest to mean 7.59 mm^2 under stress (n.s.) in participants with T1D. There was no significant difference in the results for PWV, mean 4.84 m/s ± 0.68 in control participants and mean 5.33 m/s ± 1.47 in participants with T1D and the results for CIMT, mean 0.44 mm ± 0.03 in control participants and mean 0.46 mm ± 0.03 in participants with T1D.

Conclusion

Our study demonstrated the feasibility of the IHE-MRI technique as a novel, non-invasive method of the study of vascular endothelial function that can be used in children and adolescents. Preliminary results suggest reduced elasticity of coronary vessels in children with long standing diabetes mellitus. Further studies are needed to enable a deeper understanding of the effects of T1D on vascular disease. Improved knowledge and methodologies in this area will improve surveillance and care as targeted interventions for T1D patients can be initiated in a timely manner to reduce cardiovascular morbidity and mortality.

Key words

Type 1 diabetes mellitus, endothelial function, children, isometric handgrip exercise, MRI

Introduction

Type 1 Diabetes mellitus (T1D) is a chronic disease characterized by immune-mediated beta-cell destruction requiring livelong insulin therapy. T1D is most often diagnosed in young children (2-3 years-old) and pre-teens and early adolescents (8-12 years-old). The incidence and prevalence of T1D in children and adolescents <16 years is rising and incidence rates vary across countries[1]. Despite newly emerging treatment modalities[2], high rates of mortality from acute and chronic complications persists[3] and both disease duration and elevated glycated hemoglobin (HbA1c) levels are associated with increased cardiovascular morbidity and all-cause mortality[4]. The Diabetes Control and Complications Trial (DCCT) has used magnetic resonance imaging (MRI)[5] to investigate cardiovascular effects. Endothelial dysfunction and intima media lesions are distinct yet closely related to vascular injuries as structural arterial wall alterations are linked to arterial stiffness and systemic arterial hypertension. Notably, end-stage lesions (e.g. atherosclerotic plaques) have been observed in children and young adults[6]. Clinical detection of early stages of vascular disease like endothelial dysfunction and arterial stiffness due to intima media lesion is therefore paramount, especially in subjects with risk factors including obesity, hypertension, and a positive familial history. Importantly, the availability and effects of early secondary prevention accentuate the need for non-invasive vascular studies to detect early or advanced stages of vascular disease in children and young adults.

Measurement of the carotid intima media thickness (CIMT) with B-mode ultrasonography[7] is one of the most common used methods of vascular imaging studies and has been extensively studied in adults with diabetes[8] and several reports suggest the presence of increased CIMT values in a large proportion of children with T1D[9]. Another method which has been used to asses aortic wall stiffness is aortic pulse wave velocity (PWV)[10,11,12]. Recent studies point to the utility of assessing arterial stiffness by PWV, in children with varied congenital heart and vascular malformations[13,14,15]. These studies show stiffer, less compliant thoracic aortas and impaired arterial stiffness in adolescents with T1D. Both CIMT and PWV are the standard non-invasive methods for investigating vascular effects of diabetes in children and adolescents.

Recently, Hays et al. explored the use of MRI to measure cross-sectional coronary area and blood flow changes in response to isometric handgrip exercise (IHE)[16] – a technique previously shown to be useful for assessing the effect of ischemia on myocardial metabolism[17]. Indeed, *in vivo* studies demonstrate the vasomotor response to IHE are primarily mediated by nitric oxide[18] and therefore, reflect endothelial function[19]. The feasibility of the IHE-MRI technique to assess vasomotor reactivity in coronary arteries, was demonstrated by reproducible reduced blood flow and dilatation in patients with coronary artery disease[20,21]. These results suggested that MRI is capable of distinguishing area differences in the order of 0.2-0.3 mm^2 (i.e. 3-4% difference for a 3 mm baseline diameter). Radial MRI with sufficiently high signal-to-noise ratio is thus adequate for measuring area differences in the range of previously reported endothelium dependent vasomotor response in healthy adult subjects (10-25%). Further, these results also indicated that the smallest detectable area difference with radial MRI was largely independent of pixel size in the resolution range investigated[22]. As coronary artery diameter ranges from 2 mm in healthy infants to 5 mm in healthy teenagers[23]. However, evaluation of the endothelial function by fMRI has, to the best of our knowledge, not been studied in children.

This study aimed to test the feasibility of using IHE-MRI in children and adolescents and to assess endothelial function in young patients with and without T1D. Secondarily we sought to compare IHE-MRI results with the standard CIMT and PWV techniques.

Methods and material

This investigator-initiated, single-center pilot study was an unblinded parallel trial of IHE-MRI versus CIMT and PWV examining blood vessel function in young children (pubertal and prepubertal) at the University Hospital of Lausanne (CHUV) in Switzerland.
The study protocol was approved by the institutional review board, the *Commission cantonale (Vaud) d'éthique de la recherche sur l'être humain* (CER-VD 213/15) and was conducted in accordance with the principles of the Declaration of Helsinki. Prior to study enrollment, patients and their parents/legal representatives received oral and written information about the study. Written informed consent was obtained from parents/guardians and assent was received from participants aged 14 years and older.

- ***Study participants***

Two groups of children, healthy community-dwelling children on no medications and patients with T1D (at least 5 years in duration).

Participants ranged in age from 12-18, exhibiting Tanner II or greater pubertal development. Exclusion criteria including smoking, obesity (BMI >97th percentile), systolic or diastolic hypertension (>90th percentile), dyslipidemia (pathological HDL/total cholesterol), any inflammatory process. Participants' characteristics, such as age, duration of diabetes, insulin therapies and recent Hb1AC levels were recorded for children with T1D. On the day of the exams, height, weight, heart rate and blood pressure were measured.

- ***Imaging protocol***

T1D patients removed technical material (e.g. insulin pump, continuous glucose measurement (CGM) devices) prior to imaging. This was planned in advance with the families in order to minimize the study impact on T1D management. CIMT and PWV were performed by the same operator (YM) and IHE-MRI by another operator helped by a technician (different technicians according to hospital schedule). All IHE-MRI analysis were performed by the same collaborator (JY). The exams took place in the afternoon, at least one-hour postprandial.

Vascular function was studied in all participants via CIMT and PVW for comparison with IHE-MRI. Each subject was examined by ultrasound using the Philips iE33 (Philips Medical, Netherland) echocardiograph with a linear L11-5 transducer to measure c-IMT. The data was saved on our digital archives system (Xcelera, Philips Medical Netherlands) and analyzed off-line with automated measurement (QLAB software, Philips Medical Netherlands). Images were acquired following the standards of the American Heart Association (AHA)[24]. IMT of the posterior wall was measured in three portions of the left and right carotid arteries: common carotid, internal carotid and carotid bulb, in two different angles of insonation. Based on these measurements, the maximum average value of carotid IMT was calculated and expressed in mm ± standard deviation. PWV was measured with the Sphygmocor CPV System (AtCor Medical, Sidney, Australia)[25]. The tonometer was applied at the common carotid artery and the femoral artery. Pulse wave was recorded simultaneously at both locations using electrocardiogram. The latter was chosen as time of reference, enabling the device to determine the transit time of the pulse wave. The distance between the two sites is used to calculate PWV. The device has fully-automated calculations and expresses an index of measurement quality. Per manufacturer recommendations, measures with an index < 74% were not considered. The PWV value given by the apparatus was expressed in meter per second (± standard deviation).

- ***Isometric Handgrip Exercise -Magnetic Resonance Imaging (IHE-MRI)***

Coronary endothelial function was assessed by MRI with imaging studies done at baseline (at rest) and during IHE (under stress). Maximum grip strength was determined using an MRI-compatible dynamometer (Grip Force Fiber Optic Response Pad, Current Designs Inc., Philadelphia, USA) prior to baseline imaging. Following baseline imaging, IHE-MRI commenced. Handgrip exercise was started one minute before the data collection. Each subject held the handgrip exercise at 30% of his or her maximum grip strength for about 4 minutes and images were obtained during the exercise. The handgrip was combined with custom-written MATLAB software that projected a real-time feedback enabling participants to visually observe grip strength effort while in the magnet bore enabling participants to adapt/maintain a constant grip force (30% of maximal effort). Subjects were examined in the supine position and all cine images were acquired during end expiratory breath hold to minimize respiratory motion artifacts.

Bright blood cine MR images were acquired perpendicular to a linear segment of the right/left coronary artery. We used this new technique to measure the changes of diameter of the coronary in response to handgrip exertion, combined to a non-invasive measure of blood pressure and heart rate to determine the rate-pressure product[26,27] and evaluate the effect of handgrip.

• ***Statistical analysis***

All data were coded before statistical analysis. Paired and non-paired Student-test analysis were performed as appropriate. GraphPadPrim Software was employed to chart data and compare group results. *P* values <0.05 were considered statistically significant.

Results

The study included 16 healthy children and 7 children with T1D. The characteristics of the study population are described in Table 1. The control group included 16 healthy volunteers aged 14.2 ± 2.4 years, with mean BMI 20 ± 3 kg/m^2. The T1D group included 7 children aged 14.8 ± 1.9 years, with mean BMI 21.8 ± 2.9 kg/m^2. The mean diabetes duration was 10.7 ± 3.1 years, mean total daily insulin 0.9 ± 0.2 U insulin/kg/day, mean HbA1c 9.3 ± 1.1 %. All CIMT and PWV examinations were successfully performed and IHE-MRI examinations were successfully performed in 10/16 (62%) of healthy participants and 6/7 (85%) of patients with T1D. There were no adverse events.

Both CIMT and PWV values were in the expected age-appropriate reference range and no differences were observed between the groups (Table 2).

In healthy controls, the mean CIMT and mean PWV were 0.44 ± 0.03 mm and 4.84 ± 0.68 m/s respectively consistent with normal ranges in prior reports[28,29,30]. In children with T1D, mean CIMT (0.46 ± 0.03 mm) and mean aortic PWV (5.33 ± 1.47 m/s) were within a normal range, slightly higher than in healthy study participants yet not statistically significant likely due to reduced sample size.

The IHE-MRI was feasible for children and young adults as evidenced by the fact that good-very good vascular image quality was obtained in 10/16 (62%) of healthy participants, and 6/7 (85%) of patients with T1D (Figure 2). IHE-MRI was effective imaging technique in this population as significant changes of blood pressure and rate pressure product were identified (Figure 1), confirming the usability of the IHE-method to induce vascular reaction in children and adolescents.

- ***Comparison of IHE-MRI: T1D versus healthy controls***

Among good quality images, a significant increase in coronary cross-sectional area was observed in healthy controls (mean: 5.4 mm^2 at rest to 6.39 mm^2 under stress, 18.8% ± 10.7%, p=0.0004), consistent with the expected vasodilatory effect of the IHE.

In contrast, no significant difference in average coronary cross-sectional area difference between resting state and during IHE was observed in children with T1D (mean: 7.17 mm^2 at rest to 7.59 mm^2 under stress, 10.5% ± 28.1%, n.s.). Though a small sample size, these data suggest a blunted endothelium dependent response (Figure 3).

Technical reasons for low quality IHE-MRI were varied: poor ECG triggering (n=2), increased heart rate (n=2), inaccurate planning of slice position, increased magnetohydrodynamic effects and anatomical and physiological variations (e.g. coronary artery size), absence of quiescent period during diastole, as well as noncompliance of subjects (n=1 each).

Discussion

This pilot study demonstrates the initial feasibility of using an IHE-MRI technique in children and adolescents with/without T1D. Compared to baseline measurement, the differences in blood pressure and rate pressure product elicited during handgrip suggest the utility of this non-invasive technique as surrogate marker of endothelial vascular function in children and adolescents. High-quality IHE-MRI images could differentiate coronary areas in stressed versus rest conditions – not previously reported in a pediatric population.

CIMT values and PWV values were in the expected normal range and no differences were found between control and T1D groups in states of exertion using the handgrip. In contrast, IHE-MRI technique revealed differences between groups in terms of area changes (baseline vs. stressed) pointing to an early impact of diabetes on endothelial function. The present pilot results show high variance and draws attention to several technical issues: The accuracy and precision of the self-gated signals was worse during exercise than at rest. These observations raise the question if this results from the increase in heart rate under stress. Images were indeed more blurred under stressed conditions compared to those at rest (baseline). It is conceivable that the increased cross-sectional area under stress is due to physiological vasodilation, and not a result of increased of vessel blurring. Hays and colleagues demonstrated that vasodilation during handgrip exercise only occurs in healthy adult subjects in control conditions with standard ECG-STD acquisitions and not during infusion of L-NMMA to inhibit nitric oxide.[31] Additional studies are needed to clarify questions of imaging definition by including adult participants with larger coronary arteries.

Imaging quality was influenced by the act of using the handgrip. Indeed, it is a difficult task to exercise while lying on one's back. Many participants moved their upper body during exertion using the handgrip. Thus, brief orientation/training on handgrip use without moving the upper body or the arm before entering the MRI seems relevant and necessary. This also includes training young patients to hold their breath during imaging. The IHE protocol employed a unilateral handgrip. An alternative may be to develop a bilateral, symmetrical handle that is fixed to the MRI bed – not for measuring grip strength per se, but rather to limit upper limb movements. This would entail updating the IHE-MRI protocol to adapt it for children.

These preliminary pilot imaging results suggest reduced endothelial function in children with T1D compared to healthy controls. This was explored with an IHE-MRI protocol and not with standard exams like CIMT and PWV. There are several limitations to this study including small sample size and the fact that high quality images were not obtained in 100% of participants. One possible bias is that investigators were not blinded to the participants' condition and that normal dilatation with handgrip was expected in healthy controls while milder effects were anticipated among children with T1D. As this was a pilot feasibility study, it is not powered to detect significant differences, and further studies are needed to confirm this finding and to relate it to disease duration and severity.

- ***Conclusion***

These data point to a potential non-invasive breakthrough for early detection and screening of coronary vascular complications in high risk populations such as children and adolescents with T1D. The non-invasive IHE-MRI technique described herein is feasible and produces high-quality images. Results suggest differences in vascular dilatation during handgrip in T1D children and adolescents compared to healthy controls not identified by the clinically available CIMT and PWV techniques. This novel IHE-MRI method could allow clinicians to visualize vascular effects and changes in vascular disease leading to novel therapeutic strategies and improved care.

Compliance to ethical standard

The study was approved by the ethical committee of the University of Lausanne on July 9th 2015, under approval number 213/15.

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Conflict of interest

The authors declare that they have no competing interests.

Informed consent

Informed consent was obtained from the child and representative.

Authors' contribution

GZ, SSV and MH wrote the protocol, screened patients, performed the clinical study and wrote the first manuscript of the article. JY performed MRI, analyzed and interpreted the data. YM performed vascular ultrasound studies analyzed and interpreted the data. GZ, MH and AAD created and edited figures and tables. MS and MH designed and supervised the study and interpreted the results. All authors made contributions in editing and revising the article and approved the final version.

Table 1

	Healthy Controls (n=16)		**Children with type 1 diabetes** (n=7)		***p*-value**
	mean	**SD**	**mean**	**SD**	
Age (years)	14.2	2.4	14.8	1.9	0.53
Height (SDS)	0.49	0.81	-0.09	1.35	0.21
Weight (Kg)	53.8	13.6	57.7	11.1	0.51
BMI (SDS)	0.16	0.88	0.6	1.03	0.31
Pubertal stage (Tanner)	3.4	1.4	3.9	1.2	0.42
Heart rate at rest (/minute)	72.8	11.1	85	6.9	0.014
Mean systolic arterial pressure at rest (mmHg)	110.6	9	110.7	10.4	0.98
Mean diastolic arterial pressure at rest (mmHg)	64.9	8.8	66.1	12.4	0.793
Duration of Diabetes (years in decimals)	n/a	n/a	10.7	3.1	n/a

Table 2

	Healthy controls (n=16)		Children with type 1 diabetes (n=7)		p-value
	Mean	SD	Mean	SD	
Rate pressure product Rest (bpm^xmmHg)	8248.0	1549.0	9478.5	1423.4	0.170
Rate pressure product Handgrip (bpm^xmmHg)	11314.9	2356.5	11698.5	886.8	0.757
Pulse Wave Velocity (m/s)	4.84	0.68	5.33	1.47	0.285
Carotid Intima Media Thickness (mm)	0.44	0.03	0.46	0.03	0.173
Isometric Handgrip Exercise-MRI change of area (%)	18.84 (n=10)	10.72	10.5 (n=6)	28.1	0.013

Figure 1
Comparison of rate pressure product change in the 2 groups

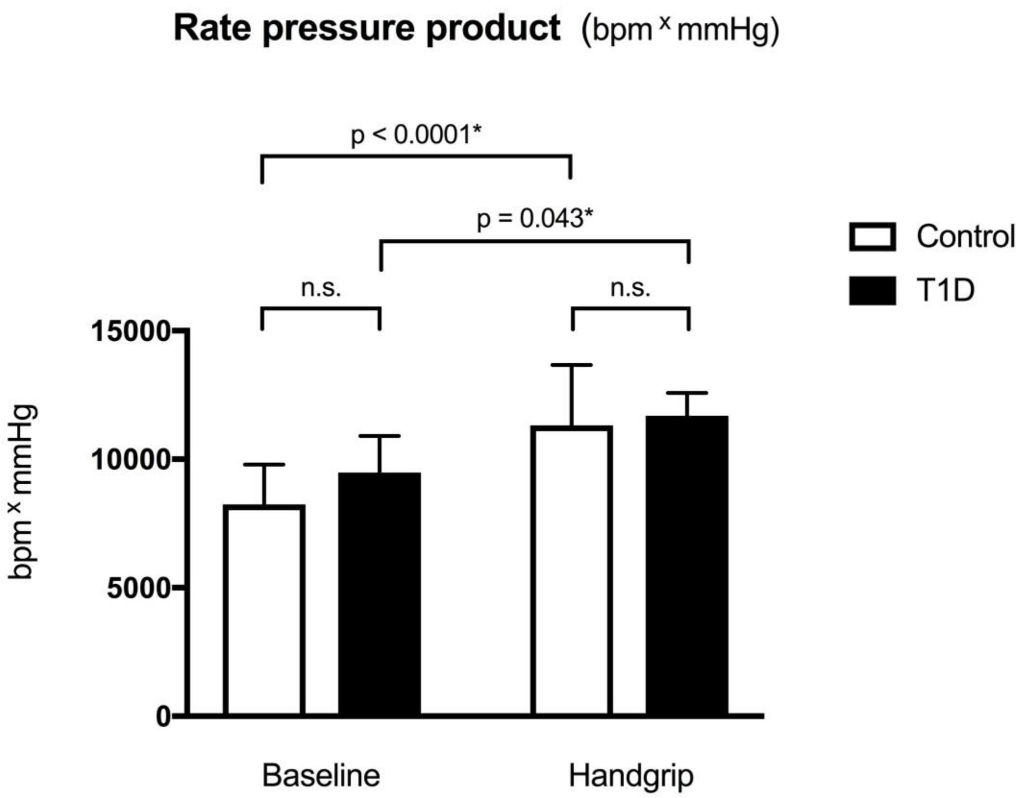
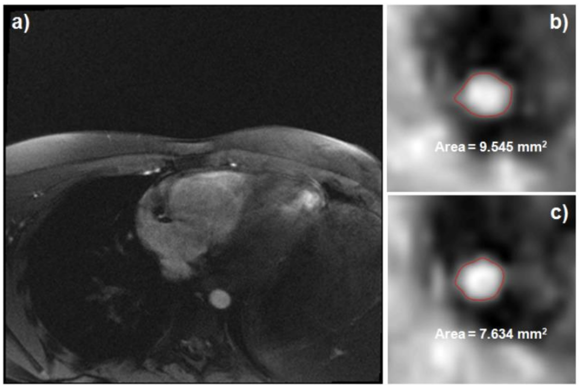
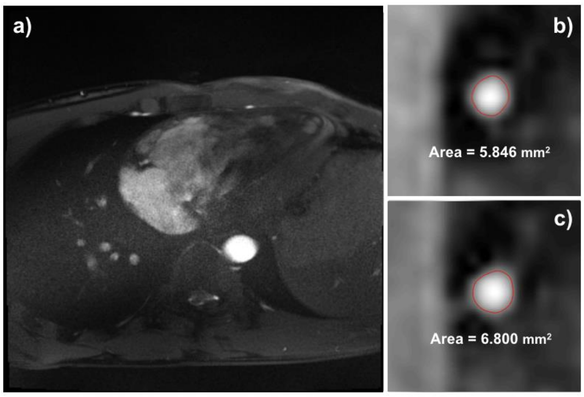


Figure 2

Representative MR images during measurement of coronary vasomotor response of the right coronary artery (RCA) to handgrip exercise. A) Left panel: child with T1D, B) Right panel: healthy control. (a) A double oblique scout scan obtained in parallel to the RCA. Cross-sectional images of the RCA acquired at rest (baseline) (b) and during isometric handgrip stress (c).

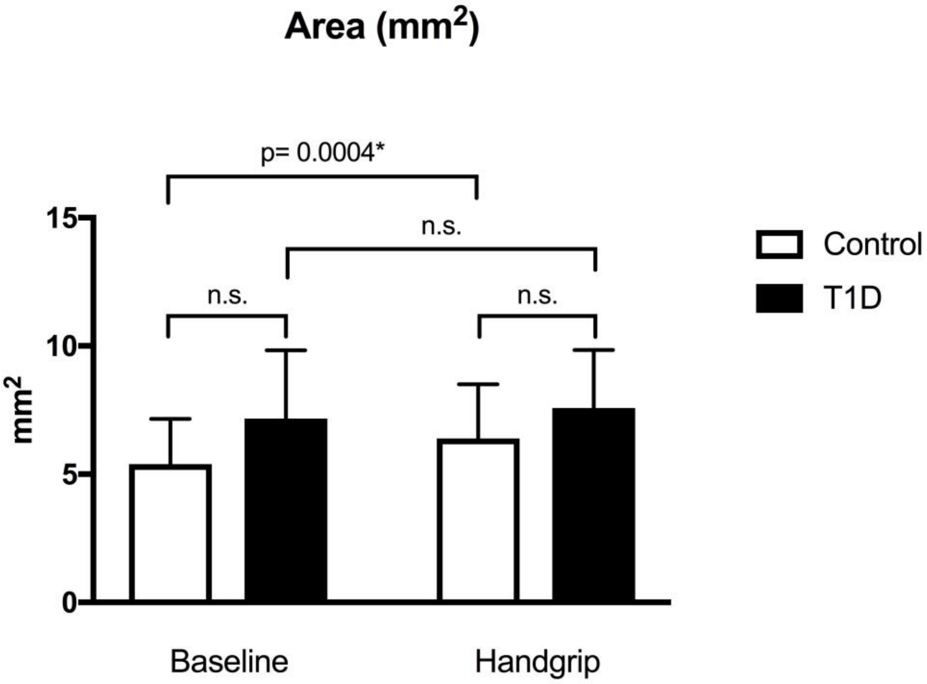


A)



B)

Figure 3
Comparison of area change in the 2 groups



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