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# **Cardiovascular disease with type 1 diabetes: A review of epidemiological data and underlying mechanisms**

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## **Abstract**

Cardiovascular disease (CVD) is highly prevalent in patients with type 1 diabetes (T1D) and a major cause of mortality. CVD arises earlier in life in T1D patients and is responsible for a significant reduction of at least 11 years' life expectancy. Also, the incidence of CVD is much more pronounced in patients with T1D onset at an earlier age. However, the factors responsible for increased atherosclerosis and CVD in T1D are not yet totally clarified. In addition to the usual cardiovascular (CV) risk factors, chronic hyperglycaemia plays an important role by promoting oxidative stress, vascular inflammation, monocyte adhesion, arterial wall thickening and endothelial dysfunction. Diabetic nephropathy and cardiac autonomic neuropathy are also associated with increased CVD in T1D. In fact, the CVD risk remains significantly increased even in well-controlled T1D patients who have no additional CV risk factors, indicating that other potential factors are likely to be involved. Hypoglycemia and glucose variability could enhance CV disease by promoting oxidative stress, vascular inflammation and endothelial dysfunction. Furthermore, even well-controlled T1D patients show significant qualitative and functional abnormalities of lipoproteins that are likely to be implicated in the development of atherosclerosis and premature CVD. In addition, recent data suggest that a dysfunctional immune system, which is typical of autoimmune T1D, might also promote CVD possibly through inflammatory pathways. Moreover, overweight and obese T1D patients can manifest additional CV risk through pathophysiological mechanisms resembling those observed in type 2 diabetes (T2D).

**Keywords:** Cardiovascular disease; Hyperglycaemia; Lipid; Type 1 diabetes; Atherosclerosis

The high prevalence of cardiovascular disease (CVD) among patients with type 2 diabetes (T2D) has been widely recognized and extensively discussed over the past several decades. Yet, the risk of CVD is also greatly increased in patients with type 1 diabetes (T1D) and a major cause of mortality in such patients [1–3]. Indeed, the incidence of T1D is rising worldwide with an earlier age of onset, leading to longer durations of disease exposure and even greater risk for premature CVD. Over the past few decades, mortality due to renal disease and acute metabolic complications has fallen, leaving a much larger number of patients who are potential victims of CVD, which is now becoming an important issue in T1D.

The present review presents the epidemiological data showing an augmented incidence of CVD in T1D patients and discusses the pathophysiological mechanisms involved in the premature atherosclerosis observed in T1D. As these data reveal, the pathogenesis of CVD differs somewhat between T1D and T2D, and various specific factors, such as recurrent hypoglycaemia, glucose variability and masked lipid abnormalities, could be playing a significant role.

## **CVD risk is significantly increased in T1D**

### ***Increased cardiovascular (CV) morbidity***

All epidemiological studies have clearly indicated that CV risk is significantly increased in patients with T1D. A large observational study performed in Scotland between 2005 and 2007 in T1D patients aged  $\geq 20$  years showed that their age-adjusted incidence rate ratio (IRR) for the first CVD event, vs the non-diabetic population, was 2.34 for men and 3.02 for women [4]. A study carried out between 1992 and 1999 using the UK General Practice Research Database (GPRD) reported a hazard ratio (HR) of 3.6 for T1D men and 7.7 for T1D women (mean age 33 years in both groups) for major CVD (myocardial infarction, acute coronary heart disease death, coronary revascularization, stroke) [5]. Data resulting from a meta-analysis involving 26 studies reported a

significant increase in the incidence rate of coronary disease of 5.62 (4.30–7.34) in T1D men and of 11.32 (8.79–20.19) in T1D women compared with the non-diabetic population [6] (Table I).

In addition, the age-adjusted IRR for a first CV event is much higher in younger patients, thereby indicating an earlier onset of CVD in T1D patients. For instance, in the Scottish registry linkage epidemiological study [4], such an age-adjusted IRR was 1.71 in men aged  $\geq 70$  years vs 4.80 at age 20–39 years, and 1.85 in women aged  $\geq 70$  years vs 5.48 at age 20–39 years. In the UK GPRD study [5], the relative HR for a major adverse CV event (MACE) was 2.3 in men aged 65–75 years compared with 11.3 in men aged  $< 35$  years, and 4 in women aged  $> 75$  years compared with 15.4 in women aged 35–45 years .

### ***Increased CV mortality***

All studies have clearly indicated, in T1D patients, not only an increased incidence of CV events, but also an increased CVD mortality (Table I). One large observational US study based on the Allegheny County (Pennsylvania) childhood-onset (age  $< 18$  years) type 1 diabetes registry [7], performed in 1075 T1D patients with a mean age of 42.9 years diagnosed between 1965 and 1979, reported a standardized mortality rate (SMR) of 13.2 in T1D women and 5.0 in T1D men compared with an age-, gender- and race-matched general population group; in that study, CVD was the main cause of death (40%). Similarly, data from the Norwegian Childhood Diabetes Registry [8] for a population of 1906 T1D patients diagnosed between 1973 and 1982, with a mean follow-up duration of 24.2 years, indicated an SMR for CVD of 11 for men and 10 for women as well as, for ischaemic heart disease, 20.2 for men and 20.6 for women, compared with an age- and gender-matched population. In the study based on the UK GPRD, the HR for CV mortality was 5.8 in T1D men and 11.6 in T1D women vs comparison groups [5]. In a study involving 2544 T1D patients without albuminuria from the Finnish Diabetic Nephropathy Study (FinnDiane) cohort [9] with a

median age of 36.3 years, over a median follow-up duration of 14 years, ischaemic heart disease was the main cause of death, with a mortality rate ratio of 4.34 compared with age- and gender-matched controls without diabetes. A prospective study performed in Scotland [1] of 24,691 patients with T1D aged  $\geq 20$  years, with a median age of 43.0 years and duration of diabetes of 18.6 years, revealed an estimated reduction in life expectancy of 11.1 years in men and 12.9 years in women; the largest part of the reduced life expectancy was related to CVD (44.9% in men, 42.4% in women). A nationwide registry-based cohort study performed in Sweden [2] and including 27,195 T1D patients and 135,178 matched controls, with a median follow-up of 10 years, showed significantly increased CV mortality with an adjusted HR of 7.38 in patients with diabetes onset at age  $< 10$  years vs 3.64 in those whose diabetes onset was at age 26–30 years. This increased mortality led to a dramatic reduction in life expectancy of 14.2 and 17.7 years in men and women, respectively, with diabetes onset at ages  $< 10$  years vs 9.4 and 10.1 years in men and women, respectively, with diabetes onset at ages 26–30 years [2]. Finally, in a Danish study involving 4821 T1D patients aged 33–57 years followed from 2002 to 2010, CVD was the main cause of death (31% and 30% of men and women, respectively) [3].

### ***Effects of childhood onset***

Thus, it is important to note that the incidence of CVD is much more pronounced in T1D patients with early diabetes onset. In the nationwide registry-based Swedish study [2], the adjusted HR for CVD was 11.44 in patients with T1D onset at age  $< 10$  years vs 3.85 in those with an onset at age 26–30 years; in addition, the adjusted HR for coronary heart disease (CHD) was 30.50 in patients with a T1D onset before age 10 years compared with 6.08 in T1D patients whose onset was at age 26–30 years (Table I). In that study, a diagnosis of T1D before 10 years of age resulted in a loss of 16.0 life-years compared with 9.8 life-years when T1D onset was at age 26–30

years [2]. It has already been mentioned, in the Scottish population by age 20 years, the estimated loss in life expectancy with T1D is, in absolute terms, 11.1 years in men and 12.96 in women, with 41% of deaths related to CVD [1].

### ***Effects of diabetic nephropathy***

Diabetic nephropathy has been associated with an increased CV risk independently of other factors. In the Pittsburgh Epidemiology of Diabetes Complications (EDC) Study [10], albumin excretion rate was an independent risk factor for major atherosclerotic events. In the Steno Type 1 Risk Engine (Steno-Risk) study [11], both albuminuria and glomerular filtration rate (GFR) were independent risk factors for a first fatal or non-fatal CVD event (ischaemic heart disease, ischaemic stroke, heart failure, peripheral artery disease). In the European Diabetes (EURODIAB) Prospective Complications Study [12], albumin excretion rate was an independent risk factor for CHD. Data from the Swedish National Diabetes Register (NDR) [13] indicate that, in T1D, macroalbuminuria (> 200 µg/min) increases the risk of CVD by 1.52, independently of age, duration of diabetes, age at diabetes onset, HbA1c, blood pressure, smoking, total/high-density lipoprotein (HDL) cholesterol ratio and history of CVD. Analysis of the same Swedish NDR study over an 8-year follow-up found that the HR for death due to CVD was 1.91 in patients with microalbuminuria, 4.01 in patients with macroalbuminuria and 10.77 in patients with stage 5 chronic kidney disease compared with normoalbuminuria patients [14]. In the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study, macroalbuminuria was associated with CV events after adjusting for age, gender, duration of diabetes, renin-angiotensin inhibitor use, smoking, updated weighted mean HbA1c and GFR [15]. In the Coronary Artery Calcification in Type 1 Diabetes (CACTI) study, an increasing albumin-to-creatinine ratio and decreasing GFR predicted coronary artery calcification (CAC) in T1D patients [16].

During the past 20 years, CV mortality has decreased significantly in patients with T1D. Analysis of the Swedish NDR of 36,869 T1D patients and 184,110 non-diabetic controls, who were followed from 1998 to 2014, showed that CVD mortality fell by 42% in the T1D population and, likewise, by 38% in the non-diabetic population [17]. However, the reduction of fatal CV outcomes did not differ significantly between the two populations, and the risk of CV death remained 4.2-fold higher in T1D patients compared with the controls [17]. Thus, despite improvement over the last two decades, the CV risk is persistently elevated in T1D patients.

### **Increased CVD in T1D: Pathophysiological data**

The mechanisms responsible for the increased atherosclerosis and CVD in T1D patients have still not been completely elucidated. Nevertheless, in addition to the usual CV risk factors, several other factors, such as chronic hyperglycaemia and nephropathy, have been shown to be important triggers. However, as CV risk remains high even when such factors are under control, other mechanisms are likely to be involved. The culprit factors suspected of being involved in the CVD increase in T1D as underlying pathophysiological mechanisms are depicted in [Fig. 1](#). Certain underlying mechanisms promoting atherosclerosis and CVD, such as inflammation, oxidative stress and endothelial dysfunction, may be common denominators across different factors ([Fig. 1](#)).

### ***Chronic hyperglycaemia***

This is a major factor responsible for the increased CVD in patients with T1D. Data from the DCCT/EDIC Study have shown that mean HbA1c levels during follow-up were a strong independent risk factor for any CVD and for MACE, which increased in risk by 42% for every 1% increase in HbA1c [18]. In the Pittsburgh EDC Study [10], both the latest HbA1c concentration and duration of



diabetes were independent factors for MACE. In the Wisconsin Epidemiologic Study of Diabetic Retinopathy [19], the average HbA1c concentration over the 20-year follow-up period was an independent factor of CV mortality [19]. Moreover, the DCCT/EDIC Study clearly demonstrated that the reduction of chronic hyperglycaemia by intensive therapy significantly decreased the risk of MACE by 57% in T1D patients compared with those receiving conventional treatment [20].

In fact, hyperglycaemia probably contributes to CVD in T1D through several mechanisms. First, hyperglycaemia increases, in cells, the formation of diacylglycerol (DAG), a potent activator of protein kinase C (PKC). Increased activation of PKC leads to the augmented production of: (i) matrix protein (collagen, fibronectin) and transforming growth factor (TGF)- $\beta$ , which promote basement membrane thickening; (ii) proinflammatory cytokines, such as nuclear factor (NF)- $\kappa$ B; (iii) vascular endothelial growth factor (VEGF), which increases angiogenesis and vascular permeability; (iv) plasminogen activator inhibitor (PAI)-1, which inhibits fibrinolysis; and (v) reactive oxygen species (ROS), *via* activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, thereby generating oxidative stress detrimental to arterial walls [21,22]. In addition, it has been found that oxidative stress promotes endothelial dysfunction by decreasing nitric oxide (NO) synthase activity and, thus, the production of NO, a potent endothelial vasodilator [23].

Second, hyperglycaemia activates the polyol pathway, which converts excess intracellular glucose to sorbitol *via* the enzyme sorbitol dehydrogenase, with concomitant oxidization of NADPH to NADP<sup>+</sup>. Overactivation of the polyol pathway leads to a reduced intracellular concentration of NADPH, which is required to regenerate reduced glutathione. Thus, increased activation of the polyol pathway further diminishes reduced glutathione, thereby inducing or exacerbating intracellular oxidative stress with further negative consequences on arterial walls [24].

Third, chronic hyperglycaemia induces non-enzymatic glycation of proteins, resulting in the formation of advanced glycation end-products (AGEs), which interact with the arterial wall through specific receptors (RAGEs) expressed in endothelial cells, thereby contributing to atherosclerosis. AGE/RAGE interactions trigger oxidative stress and NF- $\kappa$ B activation that, in turn, generate inflammatory signalling, endothelium dysfunction and increased endothelium permeability. NF- $\kappa$ B activation increases the production, by endothelial cells, of vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1, and monocyte chemoattractant protein (MCP)-1, which all contribute to enhance the attraction and adhesion of leucocytes and monocytes [25–27]. Moreover, AGE/RAGE interactions promote the expression of endothelin-1, a potent vasoconstrictor produced by endothelial cells [28]. AGEs reduce the expression and activity of NO synthase in endothelial cells, leading to a decreased production of NO, a potent endothelial vasodilator. AGEs also promote oxidation of low-density lipoprotein (LDL) and the formation of AGE-modified LDL (AGE-LDL). These modified LDLs are taken up by macrophages, *via* the scavenger receptor pathway, that stimulate the production of proinflammatory cytokines [tumour necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1, IL-6] and the formation of foam cells and atheromatous plaques. In addition, AGEs promote thrombosis by increasing the expression of tissue factor, and reduce fibrinolysis by increasing PAI-1 expression. Furthermore, AGE/RAGE interactions stimulate the activation and proliferation of smooth muscle cells [29]. Moreover, AGEs modify the extracellular matrix: the formation of cross-linkages between AGEs and extracellular matrix proteins (elastin, collagen, glycoproteins, proteoglycans) alters their turnover, leading to extracellular matrix dysfunction and decreased arterial flexibility [25,30]. In patients with T1D, plasma levels of the AGE pentosidine have been associated with CAC [31]. Finally, higher levels of methylglyoxal, a major precursor of AGEs, were independently

associated with incident CVD in a 12-year follow-up study of T1D patients [32]. Methylglyoxal has also been found to be associated with human carotid rupture-prone plaques [33].

Some data indicate that hyperglycaemia *per se* promotes endothelial dysfunction. Also, it has been demonstrated *in vivo* that transient hyperglycaemia, induced by an oral glucose load, can rapidly suppress endothelium-dependent vasodilatation in diabetic and non-diabetic individuals, probably through an increased production of oxygen-derived free radicals [34].

### ***The usual CV risk factors***

#### Tobacco-smoking

As also found in the general population, smoking tobacco is an important CV risk factor in patients with T1D. In the DCCT/EDIC Study [18], current smoking was an independent risk factor for MACE. In the Pittsburgh EDC Study [10] that followed, for 25 years, 604 T1D patients free of CVD at baseline, smoking was an independent risk factor for total CVD and MACE. Unfortunately, too many T1D patients are smokers: such patients who smoked comprised 19% in the DCCT/EDIC Study, 23% in the Pittsburgh EDC Study and 28% in the Scottish epidemiological study [4,10,18]. In the SEARCH Cardiovascular Disease Study [35], 20% of adolescents with T1D were smokers, a prevalence comparable to that observed in non-diabetic adolescents.

#### LDL cholesterol

This proved to be an independent risk factor for any CVD or MACE in the DCCT/EDIC Study [18], and for MACE or revascularization in the Pittsburgh EDC Study [36]. However, in the EURODIAB PCS, LDL cholesterol levels were not predictive of CHD in T1D patients [12]. The reasons why LDL cholesterol was not an independent risk factor for CVD in any studies of T1D patients are as yet unclear. As discussed later in this report, T1D patients manifest significant qualitative

lipoprotein abnormalities (including modified LDL particles) that are potentially atherogenic, and the direct impact of these modified and atherogenic lipoproteins on the arterial wall cannot be excluded, as they could be somewhat masking the effects of plasma LDL cholesterol levels. In any case, the Cholesterol Treatment Trialists' (CTT) Collaboration meta-analysis found that every 1-mmol/L reduction in LDL cholesterol induced a significant 21% relative risk reduction in MACE in T1D patients [37]. This is why tight control of LDL cholesterol is highly recommended in T1D patients.

### Hypertension

Raised blood pressure (BP) is an important CV risk factor in T1D patients. Indeed, systolic BP was shown to be an independent risk factor for MACE and for any CVD in the DCCT/EDIC Study [18], for MACE in the Pittsburgh EDC Study [36) and for CHD in women in the EURODIAB PCS [12]. Many other studies have also reported a high frequency of hypertension among T1D patients. In the CACTI study [38], which enrolled 652 T1D patients and 764 non-diabetic controls aged 19–56 years, the prevalence of hypertension was much higher in T1D patients compared with the controls (43% vs 15%, respectively). In a Finnish study of T1D patients [39], 33% had sustained hypertension and 23% had masked hypertension detected by ambulatory BP monitoring. In addition, it was demonstrated in that study that masked hypertension was independently associated with increased arterial stiffness.

### ***Diabetic nephropathy***

Although the statistical association between diabetic nephropathy and CVD is obvious, the mechanisms behind this link are still not totally clear. However, microalbuminuria has been clearly linked to inflammation, oxidative stress and endothelium dysfunction [40,41]. Diabetic

nephropathy might be a marker of biological disorders promoting atherosclerosis, but the direct influence of diabetic nephropathy *per se* (particularly at the stage of microalbuminuria) on the development of CVD remains an open question.

### ***Cardiac autonomic neuropathy (CAN)***

This form of neuropathy is a significant cause of morbidity and mortality, and is associated with a high risk of cardiac arrhythmias, silent myocardial ischaemia and sudden death. An association between CAN and mortality was confirmed in a meta-analysis involving patients with diabetes [42]. An 8-year German study looking at diabetes (mostly T1D) patients with CAN compared with matching patients without CAN reported significantly increased mortality (mostly of cardiac origin) in patients with CAN [43]. Also, abnormal cardiorespiratory reflexes indicating CAN have been associated with a 2.9-fold increased mortality (mostly due to CV causes) in T1D patients after a 7-year follow-up, independently of age and diabetes duration [44]. In addition, an independent association between CAN and intima–media thickness (IMT) has been reported in T1D [45]. Augmentation of sympathetic tone, another feature of CAN, was associated with diastolic dysfunction in T1D patients [46].

Although it seems likely that CAN may be contributing to a higher risk of cardiac arrhythmias and sudden death, any direct involvement of CAN in the development of atherosclerosis has yet to be definitively demonstrated. Data from the Pittsburgh EDC Study [47] from a 10-year follow-up of T1D patients indicated that the association between diabetic autonomic neuropathy and mortality may be largely explained by associations with other diabetes complications (such as nephropathy) and increased CV risk factors (such as hypertension).

### ***Other factors***

Thus far, chronic hyperglycaemia and the usual CV risk factors do not entirely explain the increased CVD risk in T1D patients. In the Swedish NDR study [48], which followed 33,333 T1D patients for a mean duration of 10.4 years, the risk for acute myocardial infarction was, compared with age- and gender-matched controls, increased by 82% in T1D patients with HbA1c levels < 7% and no additional CV risk factors. Another analysis of the Swedish NDR [14] showed significantly increased CV mortality [HR: 2.92, 95% confidence interval (CI): 2.07–4.13] in T1D patients with a time-updated mean HbA1c  $\leq$  6.9% compared with controls, after adjusting for age, gender, duration of diabetes, birth in Sweden or elsewhere, educational level and baseline comorbidities. In that same study population [14], it was also noted that the risk of death from CVD in normoalbuminuric T1D patients was 3.95 (95% CI: 2.61–5.06) compared with the general population and that the risk of acute myocardial infarction or CHD death remained higher in T1D patients with a time-updated mean HbA1c  $\leq$  6.9% and normoalbuminuria, as evidenced by an elevated HR especially in women (HR: 3.16, 95% CI: 2.14–4.65) [49]. Thus, these data all indicate that the increased CV risk in T1D is not only due to chronic hyperglycaemia, nephropathy and the usual CV risk factors, but also to other possible factors (Fig. 1). Indeed, several additional factors are thought to promote CVD in T1D.

### **Hypoglycaemia**

The literature offers conflicting data for the potential role of hypoglycaemia in the development of CVD in patients with T1D. In the EURODIAB PCS, having severe hypoglycaemic episodes at baseline were not associated with the incidence of CVD during a median 7.3-year follow-up [50]. On the other hand, a retrospective study of 3260 T1D patients from the Research

Datalink database in the UK [51], after a median follow-up of 5.0 years, reported that hypoglycaemia was significantly associated with an increased risk of CV events (HR: 1.73, 95% CI: 1.13–2.65) in patients with no history of CVD at baseline. Gimenez et al. [52] reported that, in a retrospective analysis of a national registry for insulin pump therapy including 1550 T1D patients, severe hypoglycaemia was an independent risk factor for CVD (HR: 1.82, 95% CI: 1.08–3.08;  $P < 0.03$ ).

Several arterial abnormalities have been associated with hypoglycaemia. In well-controlled T1D patients (mean HbA1c: 6.6%), those repeatedly experiencing hypoglycaemic episodes showed reduced flow-mediated brachial dilatation, increased IMTs at carotid and femoral sites, and higher soluble ICAM-1 levels than T1D patients without repeated episodes of hypoglycaemia, thereby indicating endothelial dysfunction [53]. Similarly, it was observed that, in children with T1D, hypoglycaemia assessed during continuous glucose monitoring was associated with reduced flow-mediated dilatation [54]. In T1D patients from the DCCT/EDIC Study [55] with mean HbA1c levels  $< 7.5\%$  throughout the study, severe hypoglycaemia was an independent factor associated with an elevated ( $\geq 100$ ) CAC score.

Hypoglycaemia can promote CVD in various ways. It is known that hypoglycaemia induces QT lengthening and may therefore be responsible for acute arrhythmias in T1D. Some data suggest that the 'dead-in-bed' syndrome might be related to cardiac arrhythmias associated with nocturnal hypoglycaemia [56,57]. It has been found, in non-diabetic subjects, that hypoglycaemia during a hyperinsulinaemic–hypoglycaemic clamp test induced an acquired long QT syndrome that could be prevented by selective  $\beta$ -blockade, indicating that sympathoadrenal stimulation is the main cause of hypoglycaemia-induced QT prolongation [58]. In fact, QT prolongation during hypoglycaemia has been observed in both T1D and T2D patients [59]. In addition, it has been shown that hypoglycaemia reduces NO-mediated endothelial vasodilatation in healthy subjects

during a hyperinsulinaemic–hypoglycaemic clamp [60], as seen in T1D patients who repeatedly experience hypoglycaemic episodes [53]. Furthermore, adhesion (VCAM-1, ICAM-1, E-selectin) and proinflammatory (TNF- $\alpha$ , IL-6) molecules have all been increased during hypoglycaemia in healthy non-diabetic subjects as well as in T1D patients [60,61]. Moreover, hypoglycaemia promotes platelet activation and impairment of the fibrinolytic/thrombotic balance, as demonstrated by the increases in P-selectin and PAI-1 during hyperinsulinaemic–hypoglycaemic clamps in both healthy subjects and T1D patients [60,61].

### Glycaemic variability

Increased glycaemic variability may be involved in promoting CVD in T1D, as it has been found to be independently associated with CVD in subjects without diabetes [62]. In T2D patients, glycaemic variability has been associated with coronary artery disease severity [63], and is a good predictor of the occurrence of a major CV event 1 year after acute myocardial infarction [64]. However, thus far, no studies have investigated the relationship between glycaemic variability and CVD in T1D, although an association between glycaemic variability and CAC score has been reported in men with T1D in the CACTI study [65]. Also, there are mechanistic data to suggest that glucose variability could play a role in CVD by stimulating the release of proinflammatory cytokines, such as IL-6 and TNF- $\alpha$  [66], and triggering oxidative stress [67,68], all of which are detrimental to the arterial wall. In addition, it has been shown that glucose variability induces significant endothelial dysfunction in both non-diabetics and T2D patients [69]. In adolescents with T1D, glycaemic variability was associated with inflammation independently of average glucose concentrations [70]. Furthermore, glycaemic variability has been associated with oxidative stress markers (thiobarbituric acid reactive substances, glutathione reductase) in T1D patients receiving intensive treatment [71]. However, in one study [72], levels of urinary 15(S)-8-iso-PGF<sub>2</sub>-



alpha, another marker of oxidative stress, did not correlate with the mean amplitude of glycaemic excursion (MAGE), which assesses glucose variability. Interestingly, glycaemic variability assessed by continuous glucose monitoring was associated with CAN in T1D patients [73], although such an association has either not been reported in other studies [74] or was attenuated or abolished after adjusting for other covariates such as HbA1c, age and diabetes duration [75].

Thus, while it is possible that glycaemic variability may be involved in the increased CV risk in T1D, it has yet to be confirmed by further studies.

#### Insulin resistance in overweight/obese T1D patients

In recent years, a significant increase in the numbers of overweight and obese patients with T1D has been observed [76,77]. This growing prevalence of overweight/obesity probably reflects trends observed in the general population and is most likely due to a conjunction of genetic susceptibility and unhealthy lifestyles [78]. Overweight/obese patients present with an insulin-resistant state and features of the metabolic syndrome (MetS), such as abdominal obesity, increased plasma triglycerides and low HDL cholesterol levels [79,80]. It has also been demonstrated that T1D patients with associated MetS have an even higher CV risk compared with other T1D patients [81,82].

In the DCCT/EDIC Study, triglycerides were an independent factor associated with CVD [18]. In the EURODIAB PCS, waist-to-hip ratio and triglycerides were, in men and women, respectively, independently associated with CHD [12]. In a study of 658 T1D patients from the Pittsburgh EDC Study who were followed for 25 years, plasma triglycerides and insulin resistance (as assessed by glucose disposal rate) were both independent risk factors for CVD [10]. In the DCCT/EDIC Study, the CV benefit related to intensive glycaemic control was abolished in the T1D patients who had excessive weight gains during the initial 6.5 years of the trial [83]. These patients showed,

compared with other T1D patients, a profile of insulin resistance and significantly higher insulin doses, increased triglycerides, higher systolic and diastolic blood pressure, and lower HDL cholesterol [83].

Thus, in overweight T1D patients, there is an additional CV risk related to their insulin-resistant state together with MetS that increases their development of CVD through various mechanisms, such as increased visceral tissue, low-grade inflammation, increased triglycerides, low HDL cholesterol and reduced fibrinolysis, to name but a few [84].

#### Masked lipid disorders in T1D patients

While T1D patients with poor glycaemic control show quantitative lipid disorders such as increased levels of plasma triglycerides and LDL cholesterol, T1D patients with optimal glycaemic control show normal or even 'good' lipid profiles, with normal or slightly decreased triglycerides and LDL cholesterol and normal or slightly increased HDL cholesterol [85]. However, all T1D patients, even those with good glycaemic control, manifest several qualitative and functional abnormalities of lipoproteins that are potentially atherogenic [85]. In T1D patients, very low-density lipoprotein (VLDL) particles are enriched with cholesterol whereas LDL and HDL are enriched with triglycerides [85,86]. Triglyceride enrichment of LDL leads to the formation of small, dense LDL particles that promote arteriosclerosis [85,87]. Such modification of lipoprotein composition observed in T1D is secondary to increased cholesteryl ester transfer protein (CETP) activity, which is most likely due to peripheral hyperinsulinaemia secondary to subcutaneous insulin administration [85,88]. This idea is supported by the fact that CETP activity in T1D patients is normalized when subcutaneous insulin administration is replaced by intraperitoneal insulin administration using an implantable insulin pump that mimics the physiological portal route or following a pancreatic graft [89,90]. In addition, VLDL and LDL particles from well-controlled T1D

patients show an increased free cholesterol/lecithin ratio at the periphery of lipoproteins, which could reduce their fluidity and stability [85]. Furthermore, increased levels of oxidized LDL, which is potentially atherogenic, have been reported in T1D patients [85].

Furthermore, lipoproteins from patients with T1D, even those with good glycaemic control, show significant functional abnormalities that are likely to promote atherogenesis and CVD. It has been shown, in T1D patients, that the capacity of HDL to promote cholesterol efflux from macrophages is significantly reduced independently of glucose control [85,91]. HDL particles in T1D patients even with good glycaemic control reveal a significant reduction in their antioxidative properties [85,92]. Moreover, the endothelium-dependent vasorelaxant effect of HDL is lost in T1D patients [93], a result that could be due to the decrease in sphingosine-1-phosphate (S1P) reported in patients with T1D, as S1P promotes the synthesis of NO by endothelium [94].

Indeed, the qualitative and functional abnormalities observed in T1D patients are all likely to be involved in the promotion of CVD, as suggested by data from clinical studies [85]. For instance, in T1D patients from the DCCT/EDIC Study, independent associations between carotid IMT and small, dense LDL particles or oxidized LDL were reported [85,95,96].

#### Dysfunctional immune response?

There are data to suggest that a dysfunctional immune system, which is typical of autoimmune T1D, could also promote CVD. It was reported that cardiac myosin autoantibodies were present after myocardial infarction in 83% of T1D patients, but not in T2D patients [97]. In a median 26-year follow-up study of T1D patients from the DCCT/EDIC Study [98], high levels of cardiac autoantibodies (three cases related to myosin heavy chain 6, one case related to myosin heavy chain 7 and another related to troponin) were observed mostly in patients with poor glycaemic control ( $HbA1c \geq 9\%$ ). In addition, two or more positive cases of cardiac autoantibodies

were independently associated with high plasma C-reactive protein (CRP) levels and detectable CAC [98]. These data support the idea that a dysfunctional immune response could be accelerating the development of atherosclerosis possibly through inflammatory pathways, particularly in T1D patients who are not well controlled.

In conclusion, CVD is a major threat in T1D patients. Chronic hyperglycaemia plays an important role, and optimal glycaemic control is essential to reduce CV risk in T1D. However, CVD risk remains significantly increased even in well-controlled T1D patients and in the absence of additional CV risk factors, indicating that other potential factors are probably involved. Among such factors, hypoglycaemia, glucose variability, and qualitative and functional abnormalities of lipoproteins could be playing a role in the enhanced development of atherosclerosis observed in T1D patients. Further research is now needed to clarify the factors involved in premature CVD in T1D, to identify any potential therapeutic targets and to develop adjunct therapies to prevent CVD in T1D patients.

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## Figure legend

Fig. 1. Known and potential factors, and their underlying pathophysiological mechanisms, suspected to promote cardiovascular (CV) disease in patients with type 1 diabetes (T1D). LDL, low-density lipoprotein.

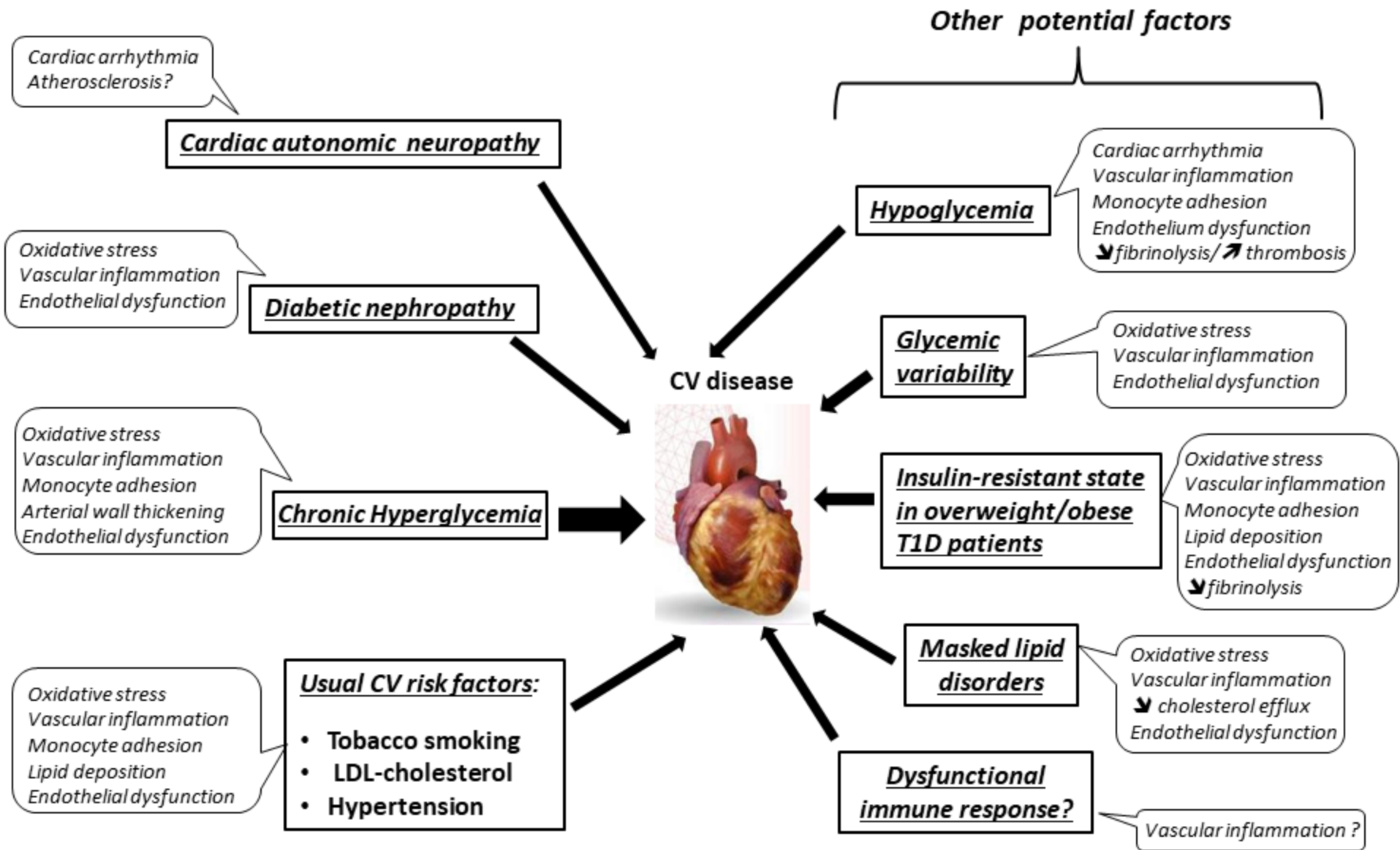


Figure 1

## **Abstract**

Background: In contrast to wearable tools (like in-shoe) sensors, lab-based gait assessment (using pressure-sensitive mats or cameras) only acquire data over short distances in non-ecological environments.

Research question: To examine the concurrent validity of a wearable ZeroWire® footswitch system (Aurion Srl, Milan, Italy) vs. the GAITRite® walkway (CIR systems Inc., NJ, USA) for recording temporal gait parameters.

Methods: We included 40 healthy participants in a prospective, single-center study. Temporal gait parameters were recorded simultaneously with the ZeroWire® and GAITRite® systems while each participant walked at three different speeds (slow (60steps/min), comfortable and maximum). To measure the validity, we calculated the interclass correlation coefficient (ICC) and the coefficient of variation (CV) for each parameter (gait cadence, stride time, step time, stance time, and single-support and double-support times). We also performed a graphical analysis using the Bland and Altman method.

Results: The footswitch system showed moderate-to-excellent concurrent validity vs. the GAITRite mat. The degree of agreement between the two assessments was greatest at the maximum gait velocity showed, with very good validity ( $ICC > 0.91$ ) seen for most parameters, whereas agreement ranged from moderate to very good for the other speeds. Independently of the gait speed, the highest levels of agreement were recorded for gait velocity, cadence, stride time, step time, and stance time. According to the CVs, both systems showed the same accuracy and double-support time was the more variable parameter.

Significance: The ZeroWire® footswitch system appears to be valid for assessing temporal gait parameters (and particularly gait cadence and stride, step and stance

times in healthy participants). It is likely to be well suited to the assessment of gait parameters under ecological conditions and in dual-task gait paradigms.

**Keywords:** gait, gait analysis, footswitch, ZeroWire®, GAITRite®.

## **1. Introduction**

Gait disorders are common features in neurological diseases and also constitute a marker of frailty in the elderly [1,2]. To allow appropriate care, assessment of a gait disorder requires objective, quantifiable, reliable data [3]. Nowadays, it is possible to use three-dimensional motion analysis systems or electronic pressure-sensitive mats for gait analysis. However, the systems have drawbacks; their use is limited to a laboratory environment, and the fact that the patient is aware of the recording period might induce measurement bias. Measuring gait parameters in a more ecological way (for example in home or street environments) brings additional clues on walking performances in daily living.

In-shoe transducers (footswitches) constitute an alternative to lab-based measurement. These are on-board systems packaged as either complete, multisensor insoles or individual sensors placed under each foot. Each type of device has a variable number of sensors. Contrary to the SMTEC® footswitch system (Sport & Medical Technologies SA, Nyon, Switzerland) [4], the validity of the 4-sensor ZeroWire® footswitch system (Aurion Srl, Milan, Italy) had not previously been evaluated.

Hence, the objective of this study was to determine the concurrent validity of the ZeroWire® system (vs. the GAITRite® mat from CIR Systems Inc., NJ) for measuring temporal gait parameters in healthy participants.

## **2. Methods**

### *2.1 Participants*



Forty healthy adults (23 women (57%); mean  $\pm$  standard deviation (SD) age: 30.6 $\pm$ 8, mean BMI = 22.1 $\pm$ 2.3kg/m<sup>2</sup>) were included. Individual characteristics of each subject can be found in Appendix A. All participants gave their written consent to participation. The study was approved by the local investigational review board and was conducted in accordance with the principles of the Declaration of Helsinki.

## *2.2. Experimental devices*

The ZeroWire® footswitch system comprises a total of four piezo-resistive pressure sensors under each foot. The trigger threshold was kept to the default setting (20% of maximum pressure). The recorded signal was binary (0 = no contact, 1 = contact), with a time resolution of 0.4ms. The four sensors were placed at the heel, at the heads of the 1<sup>st</sup> and 5<sup>th</sup> metatarsal bones, and under the distal phalanx of the hallux.

The GAITRite® system is an electronic walkway with resistive sensors that record the relative pressures under each foot. It has a spatial resolution of 1.27cm and a sampling frequency of 240Hz (i.e. a time resolution of 4ms). It has shown good validity (concurrent validity and reliability) and thus served as a gold-standard measure [5,6].

## *2.3. Experimental procedure*

Participants were asked to walk at three speeds: (i) a slow speed cued by a metronome (60 steps/min, corresponding to the usual cadence of hemiplegic patients [7]), (ii) a comfortable speed, and (iii) the highest possible (maximum) walking speed (without running). For each speed, four 10-meter walks were recorded and then averaged. Participants started 1.5 meters before the edge of the GAITRite® walkway

and stopped 1.5m afterwards, in order to avoid bias induced by acceleration and deceleration. The data generated by the GAITRite® walkway and the ZeroWire® footswitch system were recorded simultaneously. Recording began and stopped automatically for the Gaitrite, respectively at the first and last step on the mat. For the footswitches, the evaluator manually began and stopped the recording on the computer respectively just before the first and after the last step on the mat. Post-treatments to obtain the different gait parameters were done with the software of each system.

The temporal parameters recorded for each gait cycle were gait cadence and for both sides stride time, step time, swing time, stance time, and single- and double-support times. All data were expressed in milliseconds, except gait cadence (steps/min).

#### *2.4. Statistical analysis*

The quantitative data were expressed as the mean  $\pm$  standard deviation. To assess the level of agreement between the two systems, we first calculated the intraclass correlation coefficients (ICC) using a two-factor mixed model with "absolute agreement" [8]. A coefficient value of 0.30 or less indicates no agreement, a value of 0.31 or greater and 0.50 or less reflects fair agreement, a value of 0.51 or greater and 0.70 or less indicates moderate agreement, a value of 0.71 or greater and 0.90 or less indicates substantial agreement, and a value of 0.91 or greater indicates very good agreement [8–10]. Secondly, we performed a graphical analysis using the Bland and Altman method [11]. Lastly, we computed the coefficient of variation (CV), i.e. the ratio of the standard deviation to the mean, to study the accuracy of each test.

All statistical analyses were performed using SPSS software (version 22, IBM Corp., Armonk, NY).

### 3. Results

The cadence of 60 steps/min was respected by the subjects at minimal speed (Figure 1). For walking at comfortable and maximum speed, mean cadence was respectively  $107.9 \pm 6.8$  and  $141.0 \pm 15.8$  steps/min (Figure 1).

Analysis of the ICCs showed a moderate to very good agreement between the data recorded by each system (Table 1). Among the 39 temporal parameters recorded ((gait cadence + 6 lateralized parameters for each gait speed), the agreement was very good ( $\geq 0.91$ ) for 21 (53.9%), substantial ( $\geq 0.71$ ) for 8 (20.5%) and moderate ( $\geq 0.51$ ) for 10 (25.6)%. The greatest level of agreement was seen at the maximum gait speed ( $0.76 \leq \text{ICC} \leq 0.99$ ), whereas the lowest level of agreement was seen at the comfortable speed ( $0.51 \leq \text{ICC} \leq 0.99$ ). Independently of gait speed, gait cadence, stride time, step time and stance time had the highest level of agreement ( $0.86 \leq \text{ICC} \leq 0.99$ ), whereas single- and double-support times had the lowest ( $0.51 \leq \text{ICC} \leq 0.79$ ).

The Bland and Altman plot for each of the three gait speeds was drawn by pooling the data for all the temporal parameters (except gait cadence which is expressed in a different unit) (Figure 2). The mean bias remained small related to the gait parameters' time scale (4.6ms at slow speed, 8ms at comfortable speed and 4.6ms at maximum speed). Overall, the observations were suitably dispersed within the confidence interval of the mean difference between the two measurement systems, since only 4.7, 4.1 and 4.3% of observations were out of the bounds of the

confidence interval of the mean difference at respectively slow, comfortable and maximum speed. There were no systematic trends, that is no measurement error dependent of the level of the parameter assessed.

According to the CVs, the two systems had similar levels of accuracy (Table 2). For both systems, the accuracy was lower (i.e. the CV was higher) at the maximum speed and for the double-support time.

#### **4. Discussion**

This study is the first to have assessed the concurrent validity of the ZeroWire® footswitch system. The system was valid at all three gait speeds. The highest levels of agreement were observed for gait velocity, cadence, stride time, step time, and stance time. The bias between the ZeroWire® and GAITRite® systems was low relative to the gait parameters' time scale.

As in the study of the SMTEC® footswitch system [4], we found a high level of agreement between the Gaitrite and the Zerowire system for stride and step times and broader limits of agreement at comfortable and slow speeds. Relative to the SMTEC system, we found a better level of agreement for the stance time; this might be due to greater number of sensors in the ZeroWire® system, allowing a more accurate determination of heel strike and toe-off. The moderate levels of agreement for swing time and single- and double-support times observed with the ZeroWire® has been also showed with the GAITRite® [6]; it may be not specific of the footswitches and may be due to the sensors' mechanical properties. Indeed, an inertia phenomenon can create a virtual time delay between foot-off and its recording by the sensors, more obvious in parameters which have the smallest duration.

Secondly, we compared the accuracy of each system, that is an intra-device parameter studying how close the mean value of a variable is to the true value. It is noteworthy to note that both assessments showed similar values, with a lower accuracy for double stance time which is also the parameter with the shortest duration. The higher level of variability at slow speed may be due to the fact that we imposed a gait cadence. Indeed, gait at an imposed cadence can be likened to a dual task that particularly involves the primary and supplementary motor areas [12].

All taken together, no parameter showed a null or fair degree of agreement (either assessed by ICCs or with the graphical method), and no inter-devices difference were observed in CVs, indicating that the ZeroWire® system has a good concurrent validity vs the GAITRite to assess gait temporal parameters. Double support time assessment seems to be less accurate but it was observed with both tools. In a clinical point of view, it is noteworthy that the parameters found here to have the highest validity are of great value in the description of gait impairments [7,13,14]. The use of footswitches makes it possible to assess patients in a more ecological way - possibly outdoors or in their usual home environment - and to measure the impact of dual-tasking (particularly cognitive-motor interactions) on gait parameters. Other wearable systems have been developed (such as those based on inertial sensors) but their validity in non-healthy people is subject to debate [15].

Our study had a notable limitation; we only evaluated healthy participants, i.e. people with no foot deformities. In the future, it will be necessary to assess the reliability of the ZeroWire® system in a population of patients with various neurological disease. In addition, although the 2 systems showed good agreement,

data from both systems must not be mixed in the same study since there is no guarantee that they will produce strictly identical measures.

## 5. Conclusion

The ZeroWire® footswitch system showed good concurrent validity for the evaluation of temporal gait parameters in healthy adults. It is likely to be well suited to the assessment of gait parameters under ecological conditions or in dual-task gait paradigms due to its portability.

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## Figure and table legends

**Table 1.** Intraclass correlation coefficients for each gait parameter and each speed

**Table 2.** Coefficients of variation (%) for each time parameter and each speed

**Figure 1.** Gait cadence across the 3 different conditions of speed, recorded by the GAITRite: Mean  $\pm$  SD (A) and individual values (B)

**Figure 2.** Bland & Altman plots showing the difference between the GAITRite® system and the ZeroWire® footswitch system when pooling all temporal parameters (except gait cadence) for both sides at slow (A), comfortable (B) and maximum (C) gait speed. *The black line represents the mean difference (bias) between the two systems, the red lines represent the bounds of the confidence interval for the mean difference. Green dots: stride time, blue dots: step time, red dots: stance time, purple dots: double time support, black dots: single stance time, orange dot: swing time.*

**Appendix A.** Characteristics of subjects.