DOCTOR OF MEDICINE

Pharmacological modulation of insulin resistance - benefits and harms

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Dr. Sandro Vella

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Dedicated
to my treasured wife Katia,
a beacon of love, support and inspiration,
and
to our beloved first-born son, Samuel
born on November 2nd, 2013,
a few days before submission of this MD thesis.

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Declaration

With reference to this thesis being submitted to the University of Dundee for fulfillment of the degree Doctor of Medicine (MD), the undersigned, Dr. Sandro Vella declares that:

(i) he is the author of this thesis;

(ii) all cited references have been consulted by the candidate;

(iii) this thesis records work which has been carried out by the undersigned;

(iv) this thesis has not been previously accepted for a higher degree.

Dr. Sandro Vella

25th November 2013

Abstract

Aims Thiazolidinediones have been advocated as second or third line insulin-sensitizing agents in the management of type 2 diabetes (T2DM). Their widespread use has been hampered by concerns about their cardiovascular safety, including fluid retention. Metformin is established as first-line glucose-lowering pharmacotherapy in T2DM. It has also been suggested that it may have benefits in alleviating insulin resistance in type 1 diabetes (T1DM). This thesis examined: (i) cardiovascular, renal and metabolic differences between individuals with T2DM 'tolerant' or 'intolerant' of TZDs; (ii) risk factors for TZD-associated oedema in T2DM; and (iii) the potential for metformin as adjunct therapy in T1DM.

Methods (i) A small clinical study characterising TZD tolerant and intolerant individuals with T2DM; (ii) A population-based epidemiological study of TZD-induced oedema in individuals with T2DM in Tayside, Scotland (using incident loop diuretic prescription as a surrogate); (iii) A systematic review and meta-analysis of published studies of adjunct metformin in T1DM.

Results (i) During a five-day high sodium diet, two known TZD-intolerant individuals with T2DM had reductions in haematocrit, aldosterone, and diastolic BP and increases in ANP and central and peripheral augmentation indices which were outwith reference ranges derived from nine TZD-tolerant individuals; (ii) Predictors of time to loop diuretic prescription included age, body mass index, systolic BP, haematocrit, ALT and macrovascular disease but rates of this outcome did not differ by therapy: 4.3% (TZDs) vs 4.7% (other agents) [unadjusted OR 0.909 (95% CI

0.690, 1.196); p = 0.493]; (iii) In meta-analysis of nine small studies in T1DM (192.8 patient-years of follow-up), metformin was associated with a reduction in total daily insulin dose (6.6 units/day; p < 0.001) but no studies examined cardiovascular surrogates or outcomes.

Conclusions Hypotheses were generated for several potential biomarkers predictive of TZD-induced oedema but the clinical importance of TZDs as a risk factor for oedema in individuals with T2DM was questioned. As there is some evidence for the safety of metformin as an adjunct therapy in T1DM but little evidence of efficacy, larger studies are warranted.

Publications arising from this thesis

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List of abbreviations

ADA	American Diabetes Association
ADOPT	A Diabetes Outcome Progression Trial
AF-1	activation-function 1
A-F2	activation-function 2 domain
AFV	ankle-foot volume
AMP	adenosine monophosphate
AMPK	AMP-activated protein kinase
ANP	atrial natriuretic peptide
ATP	adenosine triphosphate
AUC	area under the curve
BEST	Beta-blocker Evaluation in Survival Trial
BMI	body mass index
BNP	B-type natriuretic peptide
cAI	central augmentation index
CaMKK	calcium/calcmodulin-dependent protein kinase kinase
cAMP	cyclic AMP
CAP	c-Cbl associating protein
CARDS	Collaborative AtoRvastatin Diabetes Study
CBP	CREB binding protein
CHARM	Candesartan in Heart failure Assessment of Reduction in Mortality and morbidity
CI	confidence intervals
CREB	c-AMP response element binding protein
CRTC2	CREB-regulated transcroption co-activator 2
CV	coefficient of variation
DBD	DNA-binding domain
DBP	diastolic blood pressure
DCCT	Diabetes Control and Complications Trial
DIABHYCAR	Non-Insulin Dependent Diabetes, Hypertension, Microalbuminuria or Proteinuria, Cardiovascular Events, and Ramipril
DIAMOND-HF	Danish Investigations of Arrhythmia and Mortality ON Dofetilide Heart Failure
DIG	Digitalis Investigation Group
DREAM	Diabetes REduction Assessment with ramipril and rosiglitazone Medication
EASD	European Association for the Study of Diabetes
ECG	Electrocardiogram
eGDR	estimated glucose disposal rate
EMA	European Medicines Agency

FATP1	fatty acid transporter 1
FDA	US Food and Drugs Administration
FeLi	fractional excretion of lithium
FeNa	fractional excretion of sodium
FFM	fat-free mass
FFMI	fat-free mass index
FIELD	Fenofibrate Intervention and Event Lowering in Diabetes
FM	fat mass
FOXO	forkhead box-containing protein O
FRDDNa	fractional reabsorption of distally delivered sodium
GFR	glomerular filtration rate
G6p	glucose-6 phosphatase
GLP-1	glucagon-like peptide-1
GLUT-4	glucose transporter-4
GSK3	glycogen synthase kinase-3
HbA1c	glycosylated haemoglobin
HDL-C	high-density lipoprotein cholesterol
HF	heart failure
HR	hazard ratio
ICC	intraclass correlation coefficient
IDF	International Diabetes Federation
IHD	ischaemic heart disease
InCl	inulin clearance
IRS	insulin receptor substrate proteins
LDL	low-density lipoprotein
LVEF	left ventricular ejection fraction
LVM	left ventricular mass
MAP	mean arterial pressure
MESA	Multi-Ethnic Study of Atherosclerosis
MFSU	metformin-sulphonylurea combination therapy
MO25	mouse protein 25
mRNA	messanger RNA
NHANES	National Health and Nutrition Examination Survey
NICE	National Institute for Health and Clinical Excellence
NT-pro-BNP	N-terminal prohormone of brain natriuretic peptide
NYHA	New York Heart Association
OCT	organic cation transporter
OR	odds ratio
pAI	peripheral augmentation index
PDK1	phosphoinositide dependent protein kinase

PEPCK	phosphoenylpyruvate carboxykinase
PI	phosphatidylinositol
PIP2	phosphoinositol 4,5 biphosphate
PIP3	phosphoinositol 3,4,5 triphosphate
PKB	protein kinase B
PKC	protein kinase C
PKCζ	atypical protein kinase C
PMAT	plasma membrane monoamine transporter
PPAR	peroxisome proliferator-activated receptor
Ppargc	PPAR-γ co-activator
PPRE	PPAR response element
PROactive	PROspective pioglitAzone Clinical Trial in macroVascular Events
PWV	pulse wave velocity
RECORD	Rosiglitazone Evaluate for Cardiovascular outcomes in ORal agent combination therapy for type 2 Diabetes
RR	relative risk
RXR	retinoid-X-receptor
SBP	systolic blood pressure
SD	standard deviation
SERR	standardised estimate of relative risk
SIGN	Scottish Intercollegiate Guideline Network
SIK	salt-inducible kinase 2
SOLVD	Studies of Left Ventricular Dysfunction
SRE	standardised regression estimate
STRAD	ste20-related adaptor
T1DM	type 1 diabetes
T2DM	type 2 diabetes
TBW	total body water
TK	tyrosine kinase
TZD	thiazolidinedione
UKPDS	United Kingdom Prospective Diabetes Study
UNa	urinary sodium
VEGF	vascular endothelial growth factor

Chapter 1

Introduction and literature review

Chapter 1 - Introduction and literature review

Section I - Physiological mechanisms underpinning insulin action in relation to metformin and thiazolidinedione therapy

The incidence and prevalence of diabetes is rising worldwide in epidemic proportions [1, 2]. Its associated morbidity and mortality are imposing a major burden on health care systems [1, 3, 4]. Type 2 diabetes (T2DM), accounting for over 90% of diabetes cases worldwide [5], is characterised by two major pathophysiological processes: insulin resistance (impaired responsiveness to insulin) and beta-cell failure. The hyperbolic relationship between insulin sensitivity and insulin secretion is well established; insulin secretion increases in response to a reduction in insulin sensitivity only up to the point at which the beta-cell cannot cope with the added demands such that any further increase in insulin resistance will cause a fall in insulin secretion [6, 7]. Data extrapolated from the United Kingdom Prospective Diabetes Study (UKPDS) suggest that loss of beta-cell function commences some 10-12 years before T2DM is diagnosed [8].

Insulin sensitivity varies between different ethnic groups [9] and populations, up to seven fold at any given age [10]. It is influenced by genetic susceptibilities [11], constitutional factors (such as obesity [12] and physical inactivity [13]) or both. The principal sites for insulin resistance are the skeletal muscle and the liver; adipose tissue and peripheral tissues are also implicated [14-16]. Skeletal muscle glucose transport alone accounts for 75% of the insulin-mediated glucose uptake in healthy individuals [17]. Insulin resistance has been associated with reduced expression of

insulin receptors at the surface of insulin-responsive cells [17], alterations in signal transduction pathways that are activated following insulin binding to the receptor [18], and abnormalities in glucose transport and glycogen synthesis [19, 20]. The role of leptin, adiponectin, and adipocytokines in adipose tissue inflammation, and their contribution to insulin resistance is also generating considerable interest [21].

Although type 1 diabetes (T1DM) is characterised by autoimmune beta-cell failure, insulin resistance is being increasingly recognized as an important pathophysiological feature, resulting in an association of this disease with the components of the metabolic syndrome [22-24]. The relevance of this association is further enhanced by the observation that insulin resistance is an independent risk factor for vascular complications, both in type 1 [24-32] and in type 2 diabetes [33-35]. Randomized controlled trials in T2DM have shown that a pharmacologically-mediated reduction in insulin resistance decreases the incidence of diabetes and the risk of macrovascular complications keep [36-39]. Tight glycaemic control has been shown in the Diabetes Control and Complications Trial (DCCT) to reduce rates of microvascular complications in T1DM [40].

Targeting insulin resistance and hyperglycaemia through different and complementary mechanisms, metformin and thiazolidinediones are widely used, alone or in combination, in the management of T2DM. However, the benefits of thiazolidinediones have been hampered by their association with fluid retention, bone fractures [41], and a possible association with myocardial infarction (rosiglitazone) and bladder cancer (pioglitazone) [42]. While metformin’s use in T2DM is firmly established, there is currently considerable interest in its potential in

T1DM. This review will address the issues surrounding thiazolidinedione-associated fluid retention in T2DM patients. Moreover, it will examine the evidence supporting insulin resistance in type 1 and type 2 diabetes and benefits associated with the use of metformin in T2DM.

1.1 The insulin signalling pathway

The pleiotropic effects of insulin are mediated through its interaction with a signalling network of molecules that are set in motion following the hormone's binding to its receptor (figure 1.1). The insulin receptor is an integral membrane glycoprotein existing as a dimer. Each monomer contains an α- and a β chain. The α-subunits link to each other and to the β-subunits by disulfide bonds, and are located on the extracellular side of the plasma membrane [43, 44]. The β-subunits traverse the membrane, and are characterised by a tyrosine kinase (TK) enzyme domain on the cytoplasmic side [44-46]. Insulin binding to an α-subunit activates the TK domain on the β-chain, leading to autophosphorylation of the TK domains in each β-subunit. Insulin receptor substrate proteins (IRS) are then recruited to the plasma membrane through an interaction with the phosphorylated insulin receptor, resulting in phosphorylation of IRS tyrosine residues [47, 48]. Phosphorylated IRS in turn recruit additional signalling proteins.

The lipid kinase phosphatidylinositol (PI) 3-kinase binds to IRS proteins and converts phosphoinositol 4,5 biphosphate (PIP2) to phosphoinositol 3,4,5 triphosphate (PIP3) [49]. This in turn recruits peckstrin homology domain containing proteins to the membrane, altering their conformation and activating protein kinase

cascades. The best characterised of these is the phosphoinositide dependent protein kinase (PDK1) pathway. PDK is a master regulator of a number of protein kinases, including protein kinase B (PKB, also known as Akt), PKC, p90, RSK, p70, S6K and SGK [50], which in turn phosphorylate and regulate a wide variety of proteins involved in growth and metabolism. Of relevance to glucose homeostasis, PKB phosphorylates and inactivates glycogen synthase kinase-3 (GSK3) [51, 52] and forkhead box-containing protein O (FOXO) [53, 54] transcription factors. By regulating the transcription of PEPCK and glucose-6-phosphatase genes, these two transcription factors modify two important rate controlling steps in gluconeogenesis. Hepatic expression of both PEPCK and glucose-6-phosphatase is high in animal models of diabetes, and overexpression of PEPCK is sufficient to induce diabetes in animals [55].

How does insulin regulate the expression of PEPCK and glucose 6-phosphatase? During starvation, glucagon promotes the assembly of a nuclear transcription complex comprising CREB (c-AMP response element binding protein), CBP (CREB binding protein) and CRTC2 (CREB-regulated transcription co-activator 2, also known as TORC2). This complex increases the expression of PPAR-γ co-activator 1 (Ppargc1), PEPCK, glucose-6-phosphatase, and other key gluconeogenic enzymes. Postprandial activation of the PI3-PKB pathway stimulates salt-inducible kinase 2 (SIK2), which inactivates the CREB-CBP-CRTC2 complex by phosphorylating CRTC2 at Ser171 and targeting it for degradation in the cytosol [56] (figure 1.1).

Although the IRS/PI 3-kinase/PDK1/PKB pathway is considered a major pathway of insulin action, it is not the only pathway downstream of IRS. The Ras-ERK pathway

has also been elucidated. In summary, the protein complex Grb2/mSOS interacts with phospho-IRS (tyrosine residues being phosphorylated at sites distinct to those that recruit PI 3-kinase). Bound mSOS exchanges GDP for GTP on the small G-protein Ras, activating Ras [57]. This in turn activates the oncogene c-Raf, which additionally has protein kinase activity. c-Raf phosphorylates and activates MAP/ERK kinase (MEK) [58], which in turn phosphorylates and activates ERK1/2 [59]. The latter acts on multiple substrates, most of which are related to cell growth.

Insulin has also been reported to regulate several other proteins relevant to glucose homeostasis, such as Rab, atypical PKC (PKCζ,), CAP and GLUT4 (all involved in glucose transport) and PDE3, hormone sensitive lipase and ATP citrate lyase (involved in fat metabolism) [60]. In particular, the translocation of the glucose transporter GLUT-4 from the intracellular pool to the plasma membrane plays a crucial role in insulin-mediated glucose entry into skeletal muscle [61], and is thought to be mediated by PI3-kinase and its downsteam phosphorylation of PKB [62] or atypical PKC [63, 64]. In summary, insulin signalling is a complex, as yet incompletely unravelled pathway potentially prone to dysregulation or mutation at several molecular points, resulting in insulin resistance.

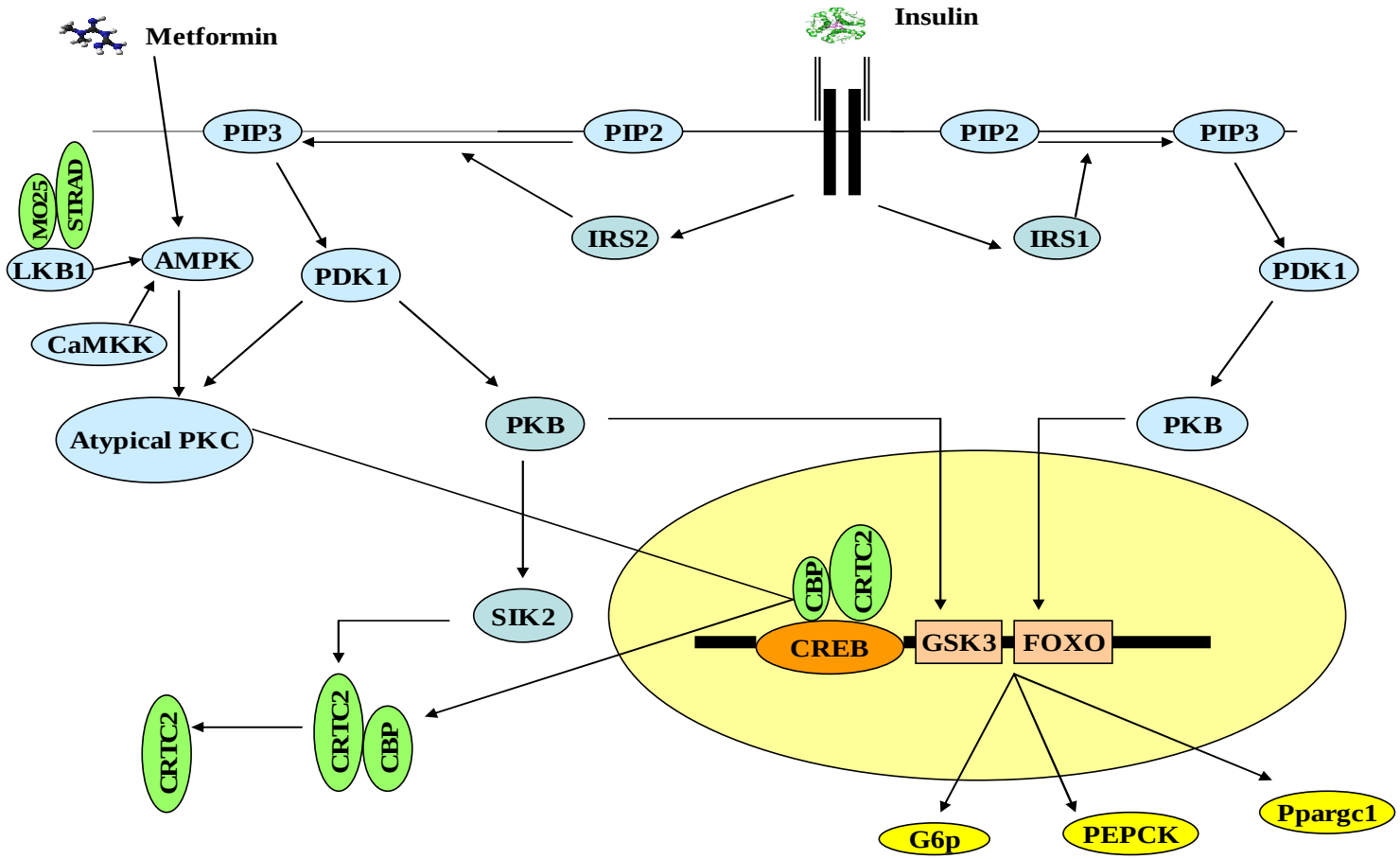
1.2 Diabetes is associated with defective insulin signalling

The molecular pathology of insulin resistance is not yet established. It is likely to result from a post-receptor defect, reducing the ability of insulin to mediate its pleiotropic actions at hepatic, skeletal muscle and adipose tissue level. Although it is

assumed that obesity predates and promotes the molecular defects, this has not been formally proven in man [60].

Insulin resistance can be generated in mice by deleting key insulin signalling molecules. Thus, a partial loss of the insulin receptor (IR +/-), combined with a partial loss of IRS1 (+/-), results in severe insulin resistance, and a greatly increased prevalence of diabetes [65]. Reduced IRS expression has been reported in association with obesity and T2DM [66]. Reduced IRS1 signalling has also been reported in human T2DM [67-69]. Phosphorylation of IRS on serine and threonine residues, as opposed to tyrosine residues (as discussed previously), reduces the interaction of IRS with the insulin receptor and downstream signalling components [70, 71] and increases the rate of IRS degradation. Serine phosphorylation has been ascribed to feedback from downstream components (eg p70S6K) and protein kinases induced by obesity, such as PKC or JNK [72-76], providing a link between obesity and insulin resistance. Several isoforms of PKC, IKK, Mtor/p70S6K and GSK-3, implicated in serine/threonine phosphorylation, are activated by free fatty acids, ceramide, TNF-α and chronic hyperinsulinaemia [71, 77, 78].

Figure 1.1 - Schematic diagram illustrating the main insulin signalling pathways regulating glycaemic control and metformin's pharmacological effects



There is evidence suggesting that the Ras-ERK pathway may be defective in at least one insulin resistant state, called polycystic ovary syndrome [79, 80], and in many young males with a BMI exceeding 29kg/m^2 [60]. Similarly, in a study of 22 normoglycaemic young men with a body mass index (BMI) ranging from 20 to 37 kg/m^2, Ruiz-Alcaraz et al. concluded that the MAP-ERK pathway (amongst other insulin signalling pathways) is defective in obese insulin resistant individuals [81], implicating that such defects predate a clinical presentation with overt diabetes.

The analysis of the intracellular insulin signalling process in man is technically problematic. Individually, insulin signalling mutations have little effect owing to considerable apparent redundancy of pathways. In summary, in humans, insulin resistance is thought to arise from the synergistic effect of multiple minor molecular signalling defects [60].

1.3 Metformin – a multifaceted therapeutic approach to insulin resistance

Metformin is most widely prescribed oral anithyperglycaemic agent worldwide, and is recommended as a first line agent in the treatment of T2DM by several national and international diabetes guidelines, such as those issued by the National Institute for Health and Clinical Excellence (NICE) [82], the Scottish Intercollegiate Guideline Network (SIGN) [83], the European Association for the Study of Diabetes and the American Diabetes Association (EASD/ADA) [84], and the International Diabetes Federation (IDF) [85]. Used for approximately 55 years in the UK (although for only 18 years in the US), metformin decreases intestinal glucose absorption, reduces hepatic glucose production by over 30% [86] and increases

peripheral glucose disposal through complex insulin-sensitizing and insulin-independent mechanisms [87].

1.3.1 Metformin and AMPK

The highly conserved energy sensor adenosine monophosphate (AMP)-activated protein kinase (AMPK) has been identified as a key modulator of the pharmacological effects of metformin [88] and thiazolidinediones [89]. AMPK is activated by a range of physiological and pathological stresses that increase the intracellular AMP: adenosine triphosphate (ATP) ratio, either by decreasing ATP generation (eg ischaemia or hypoxia) or increasing ATP consumption (eg muscle contraction). This kinase acts to restore cellular energy balance by favouring ATP generating pathways (eg fatty acid oxidation) while inhibiting ATP utilizing pathways (eg fatty acid synthesis and gluconeogenesis). This is achieved initially by direct phosphorylation of key metabolic enzymes, and in the long term by effects on gene transcription [90-92]. Additionally, AMPK is also involved in the central regulation of food intake and energy expenditure in response to hormones such as leptin, ghrelin and adiponectin [93].

AMPK exists as a heterotrimeric complex containing a catalytic subunit (α), and two regulatory subunits (β and γ) [94]. The α-subunit contains the catalytic domain, including the all important Thr172 subunit, which is phosphorylated by upstream kinases. The major upstream kinase in mammalian cells is a complex of the protein kinase LKB1 and two accessory subunits STRAD (Ste20-related adaptor) and MO25 (mouse protein 25) [95-97]. LKB1 is dependent on the STRAD subunit in order to

phosphorylate the Thr172 subunit [96]. Besides LKB1, STRAD and MO25, AMPK can also be activated by an LKB1-independent mechanism involving calcium/calmodulin-dependent protein kinase kinase (CaMKK) [98-100]. The β subunit has a glycogen binding C-terminal domain; high glycogen content exerts an inhibitory effect on AMPK through an interaction with the β-subunit in skeletal muscle, although the exact mechanism is unknown [101]. The γ subunit contains four repeats forming two tandem domains, each of which bind one molecule of ATP or AMP in a mutually exclusive manner [102]. The tandem domains bind AMP with a high degree of cooperativity [102], suggesting that the second site is inaccessible to AMP until the latter has bound to the first tandem domain. Interestingly, insulin and AMPK signalling pathways work in the same direction at the level of skeletal muscle, liver and adipose tissue, particularly for processes that regulate glucose homeostasis [103]. As with insulin, AMPK-mediated skeletal muscle glucose disposal is achieved through an increased translocation of the glucose transporter GLUT4 to the plasma membrane, although the fate of the glucose is different: glycogen synthesis in the case of insulin and glycolysis/oxidation in the case of AMPK [104, 105]. Both insulin and AMPK inhibit hepatic gluconeogenesis by repressing the expression of gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6 phosphatase (G6p) [106]. Both insulin and AMPK inhibit hormone-sensitive lipase, and hence lipolysis [107-109], albeit through different mechanisms. Thus, AMPK phosphorylates hormone sensitive lipase at Ser565, an effect that antagonises activation by cAMP-dependent protein kinase [110], whereas insulin causes phosphorylation and activation of phosphodiesterase 3B by PKB, thus lowering cAMP [111].

Metformin is thought to activate AMPK indirectly through an inhibition of complex 1 of the respiratory chain [112], causing an increase in the AMP/ATP ratio. Inhibition of the respiratory chain in the intestinal mucosa may account for the gastrointestinal adverse effects of this drug [113]. The same mechanism may also underlie the propensity of its biguanide predecessor phenformin (now withdrawn) to precipate lactic acidosis [113]. It is pertinent to point out, however, that metformin pharmacotherapy has not been associated with a significantly increased risk of lactic acidosis in a recent Cochrane review [114].

Metformin has been reported to activate AMPK in cardiac myocytes [115-117], hepatocytes [88] and skeletal muscle cells [88]. LKB1 plays a crucial role in metformin's interaction with AMPK, such that liver specific knock-out of LKB1 ablates metformin's ability to lower blood glucose in obese rodents [118]. Like insulin, metformin also stimulates the phosphorylation of CREB-regulated transcriptional coactivator 2 (CRCT2) at Ser171. This sequesters CRCT2 into the cytosol, and away from the nucleus, barring any effects on gluconeogenic gene transcription. In obese and insulin resistant individuals, CRCT2 is O-glycosylated at Ser171, blocking any benefical phosphorylation by metformin at this site [119]. Both insulin and metformin circumvent this block by activating atypical Protein Kinase C, which phosphorylates CBP at Ser436, initiating the dissociation of the CBP:CRTC2 from CREB, and targeting CREB for dissociation in the cytosol [120]. Despite these findings, the relevance of a metformin-AMPK interaction has recently been questioned, following observations that metfomin-treated mice lacking AMPK in the liver achieved comparable glycaemic control as wild-type mice [121]. Moreover, Forretz et al. observed that metformin-induced inhibition of glucose production was

higher in AMPK- and LKB1-deficient hepatocytes compared with wild-type hepatocytes, and that this inhibition correlated in a dose-dependent manner with a reduction in intracellular ATP content. This led the authors to suggest that metformin reduces hepatic gluconeogenesis through a reduction in hepatic energy state (possibly through an interaction with complex 1 of the respiratory chain), independently of any AMPK- or LKB1- related repression of gluconeogenic genes [121].

In contrast, metformin is reported to inhibit AMPK in the hypothalamus, by inhibiting low glucose-induced AMPK phosphorylation and neuropeptide-Y mRNA expression [122]. This mechanism is thought to underlie metformin's anorectic effects. Indeed, a recent study carried out on a new delayed-release formulation of metformin (newmet) concluded that higher plasma concentrations of metformin do not confer increased therapeutic efficacy. Bypassing the upper gastrointestinal tract, lowering systemic exposure and improving tolerability through its special pH-sensitive coating, newmet is reportedly able to maintain its glucose-lowering effect through an activation of nutrient receptors located on enteroendocrine cells. The latter produce key glucose-regulating hormones such as peptide YY (which signals satiety to the brain) and glucagon-like peptide-1 (GLP-1) [123]. Other studies suggest that metformin may have a deletirious effect on pancreatic beta-cell function by reducing mitochondrial ATP synthesis, a scenario that impairs responsiveness, inhibits insulin release, and possibly induces beta-cell apoptosis [124] [125].

1.3.2 The insulin-independent effects of metformin: effects on glucose absorption

The contribution of the intestine in metformin's antihyperglycaemic effects is often overlooked because of paucity of clinical data. In a study on normal 18 hour fasted mice, Wilcock and Bailey reported that metformin (administered as an intragastric bolus) decreased intestinal glucose absorption in a dose dependent manner through effects on mucosal and serosal glucose transfer, mostly in the middle portion of the small intestine [126]. Animal studies suggest that metformin delays glucose absorption, such that this occurs more distally in the gastrointestinal tract [126, 127]. Metformin administration results in the accumulation of very high drug concentrations in the intestinal wall [128]. This is accompanied by an increased utilization of glucose by the intestine, particularly through anaerobic metabolism [129-131], explaining, at least in part, the apparent shortfall in the passage of glucose from the luminal to the serosal surface of the intestine. To this effect, Bailey et al. reported that incubation of human jejunal biopsy tissue with metformin significantly increased lactate production within the tissue sample by 35%. Additionally, in a study on eight recently-diagnosed, obese, drug naïve T2DM patients, the authors showed that incident metformin administration is associated with metformin jejunal concentrations ranging from 30 to 300 times higher than plasma metformin concentrations [132].

1.3.3 Metformin and the organic cation transporter

Primarily excreted unchanged in the urine, metformin is a substrate of a number of organic cation transporters; those identified so far are organic cation transporters 1 and 2 (OCT1 and OCT2) and plasma membrane monoamine transporter (PMAT). Organic cation transporters are polyspecific transporters most commonly expressed in the liver and the kidney, where they play a role in the elimination of organic cations from the systemic circulation.[133-135]. In particular, OCT1 is thought to be a major determinant of metformin's pharmacological effects in the liver [136, 137]; passive diffusion and other transporters may account for a small portion [137]. In a transgenic mouse model, knockout of liver OCT1 virtually abolished biguanide-induced hepatic lactate production [136]. Deletion of the OCT1 gene in mouse liver reduces metformin's effects on gluconeogenesis and the drug's interaction with AMPK [137]. OCT1 polymorphisms have been reported to reduce metformin effects on the response to oral glucose, and affect serum metformin concentrations [137, 138], and may, at least partly, explain why about 40% of metformin-treated T2DM patients fail to achieve target fasting plasma glucose levels [139, 140]. Expressed in the basolateral membrane of renal tubular cells, OCT2 is implicated in the renal excretion of the drug [141, 142]. While both OCT1 and OCT2 are expressed at low levels in the basolateral membranes of entrocytes [133, 135, 143], PMAT has recently been identified as a more important metformin transporter in the small intestine, and is expressed at higher levels in the apical membrane of these cells [143-145]

1.4 Thiazolidinediones – a ‘novel’ class of insulin sensitizers

The thiazolidinediones (TZDs) rosiglitazone and pioglitazone were approved by the US Food and Drugs Administration (FDA) as pharmacological agents in the management of individuals with T2DM in 1999. Thiazolidinediones are currently recommended as second or third line T2DM pharmacotherapy by NICE and SIGN [82, 83]. The 2009 consensus statement of the EASD and the ADA did not recommend the use of rosiglitazone in view of concerns about its cardiovascular safety profile, while suggesting that pioglitazone may be used as a second line agent in specific clinical circumstances, such as ‘when hypoglycaemia is particularly undesirable’ [84]. The updated 2012 EASD/ADA recommendations, guided by the principle of *'primum non nocere'* ('first do no harm') retain a potential role for pioglitazone as a second-line add-on agent. However the authors seemingly prefer to focus on its safety and adverse effect profile [146]. Concerns about the cardiovascular safety profile of rosiglitazone, initially raised by (the much disputed) Nissen and Wolski’s meta-analysis [147], and confirmed by some [148-150], but not other [151-154] studies and meta-analyses led the US Food and Drug Administration (FDA) to issue guidance detailing the approach for acquiring, analysing and reporting the necessary safety information from all Phase II and III trials [155]. Acting upon updated meta-analyses data [156, 157], FDA restricted rosiglitazone’s use in the management of T2DM [158]. The European Medicines Agency (EMA) went further, withdrawing its marketing authorization with immediate effect in September 2010 [159]. A recent editorial has questioned the wisdom of curtailing rosiglitazone's marketing authorization, given the limitations imposed by the available medical evidence [160].

Thiazolidinediones lower fasting and postprandial blood glucose levels by increasing insulin sensitivity in muscle, fat and liver cells. This is achieved through modulation of peroxisome-proliferator-activated receptor-γ (PPARγ) activity. Troglitazone, the first widely used thiazolidinedione introduced in 1997, was withdrawn from clinical practice on account of liver toxicity [161]. The association between thiazolidinedione therapy and heart failure (HF) was reported in the same year, when Hirsch et al. described two cases of pulmonary oedema complicating the use of troglitazone in two diabetes patients with preserved left ventricular function. This clinical condition improved after the drug was discontinued [162]. Fluid retention and weight gain have since been confirmed as the principal adverse effects of rosiglitazone and pioglitazone, such that drug manufacturers do not recommend their use in patients with New York Heart Association (NYHA) functional class III or IV HF [163, 164]. Both NICE and SIGN guidelines have adopted a more stringent approach, such that they do not recommend the use of these drugs in any patient with HF [82, 83]. This chapter aims to review the current understanding of the pathophysiology of PPAR-γ agonists. Additionally, it shall discuss the clinical evidence and mechanisms underlying thiazolidinedione-induced oedema.

1.4.1 Peroxisome Proliferator Activated Receptors - a heterogenous family of nuclear receptors

The identification of the insulin-sensitizing properties of thiazolidinediones in animals and humans has generated significant interest into the mechanism of action of these drugs. Thiazolidinediones act as peroxisome proliferator-activated receptor

(PPAR)-γ agonists. Together with PPAR-α and PPAR-δ, PPAR-γ belongs to a nuclear receptor superfamily of transcription factors [165] which are activated by polyunsaturated fatty acids, prostanoids and oxidised fatty-acids found in low density lipoproteins (LDLs) [166-168]. PPAR-α, -δ and –γ are encoded on three different genes (PPARA, PPARD, and PPARG) located at chromosomes 22, 6, and 3, respectively [165]. While PPAR-δ is ubiquitously expressed [169], PPAR-α distribution is largely restricted to tissues where active fatty acid catabolism occurs. Thus, although predominantly expressed in the liver, it has additionally also been identified at moderate levels in the kidney and brown adipose tissue, and at relatively lower levels in heart and intestine [170]. It has also been localised in skeletal muscle [171]. PPAR-γ is mostly, though not exclusively, expressed in white and brown adipose tissue; additionally, it has been localised in the intestine, vascular endothelium, macrophages, pancreatic beta cells [172, 173] and skeletal muscle [174]. It is characterised by several splice variants, named PPAR-γ1 to PPAR-γ7 [175-177] , the relative distribution of which is further outlined in table 1.1.

Table 1.1 - Peroxisome proliferator-activated receptor-γ (PPAR-γ) receptor isotype distribution (adapted from [175-177])

PPAR-γ receptor isotype	*Physiological distribution*
PPAR-γ1	Mostly expressed in adipose tissue and large intestine Intermediate expression in liver, kidney and small intestine Very limited expression in muscle
PPAR-γ2	Same distribution as for PPAR- γ1, but much less abundantly expressed
PPAR-γ3	Adipose tissue and large intestine
PPAR-γ4	Macrophages
PPAR-γ5	Macrophages
PPAR-γ6	Adipose tissue
PPAR-γ7	Adipose tissue

PPARs and other class II nuclear receptors are composed of six structural regions (A to F) in four functional domains [178] (figure 1.2). The A/B region is a variable region located in the NH_2 end of the receptor. It encompasses a ligand-independent transactivation domain (activation-function 1) (AF-1) that is transcriptionally active in the absence of ligands. The ligand-binding activity of the receptor can be modified positively (in the case of PPAR-α) [179] or negatively (in the case of PPAR-γ) [180, 181] by phosphorylation [182] or sumoylation [183]. The C-region holds the DNA-binding domain (DBD), which is the most conserved domain in all nuclear receptors. It targets the PPAR to a sequence of nucleotides within the regulatory regions of responsive genes. This sequence is called the PPAR response element (PPRE) [184].

The E/F region contains the ligand binding domain and a co-activator/co-repressor binding surface [185]. X-ray crystallography has revealed that this ligand-binding domain is characterised by a large binding pocket that allows the transcription receptor to bind to a wide variety of structurally unrelated ligands [186]. The activation-function 2 domain (A-F2), located close to the C-terminal region of the receptor, is an integral component of the ligand binding domain. The binding of antagonists to AF-2 stabilises the PPAR into an unliganded state [187]. Conversely, agonists alter the structural conformation of AF-2 on binding to this domain, locking the receptor into an active conformation, which results in an increased activity of the receptor [186]. The mutable linker region D permits the rotation of DBD, connecting it the E/F region [178, 185].

Like other class II nuclear receptors, PPAR-γ are thought to exist as heterodimers with retinoid-X-receptors (RXRs) and, as discussed, bind to PPRE within the promoter domains of target genes via the DBD [178]. The unliganded PPAR-γ•RXR heterodimer is associated with a multiprotein corepressor complex that contains histone deacetylase activity. The latter inhibits nucleosome transcriptional activity. PPAR-γ receptor ligand binding results in dissociation of the corepressor complex and the recruitment of a coactivator complex containing histone acetylase activity. This in turn favours chromatin remodelling and active gene transcription [188]. PPAR-γ activation favours the differentiation of adipocytes and other cell types and the induction of lipogenic enzymes and glucoregulatory proteins. The existence of multiple PPAR-γ isoforms and their wide range of distribution may increase the diversity of ligands and their tissue-specific transcriptional responses [185].

Figure 1.2 - Structure of the peroxisome proliferator-activated receptor-γ (PPAR-γ) (adapted from [189])

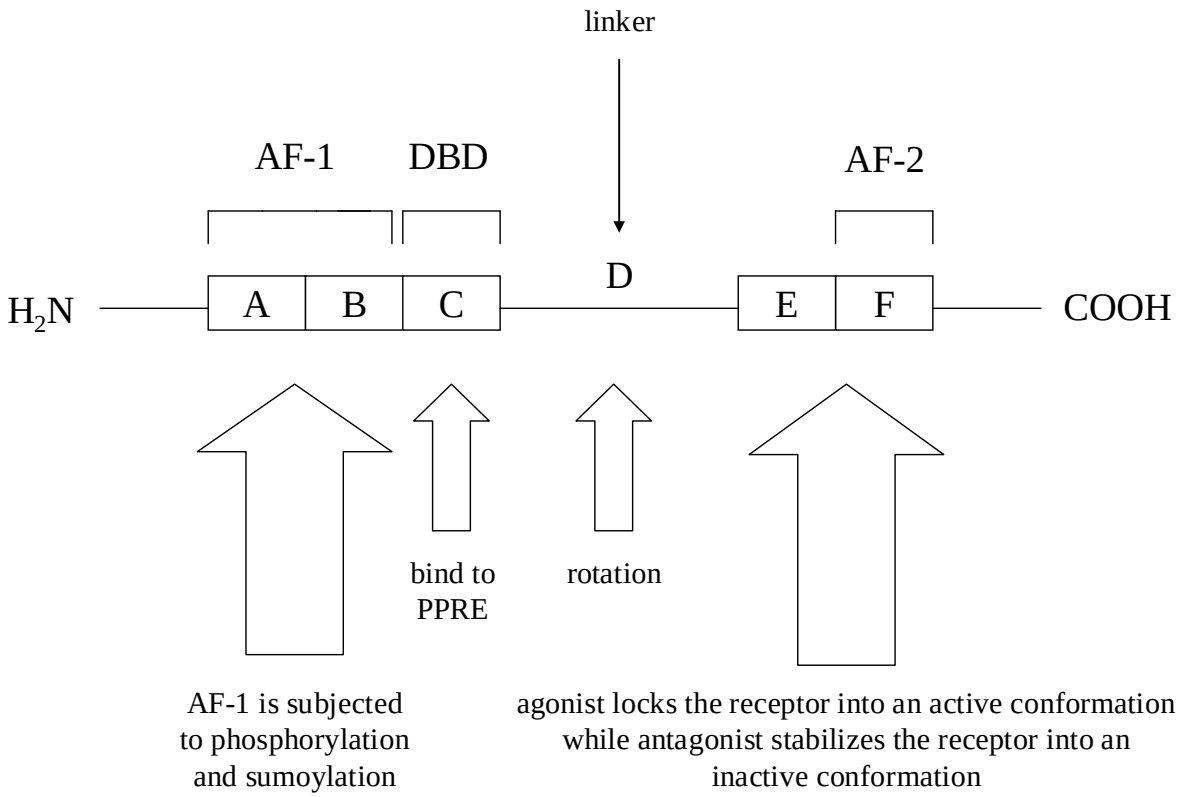
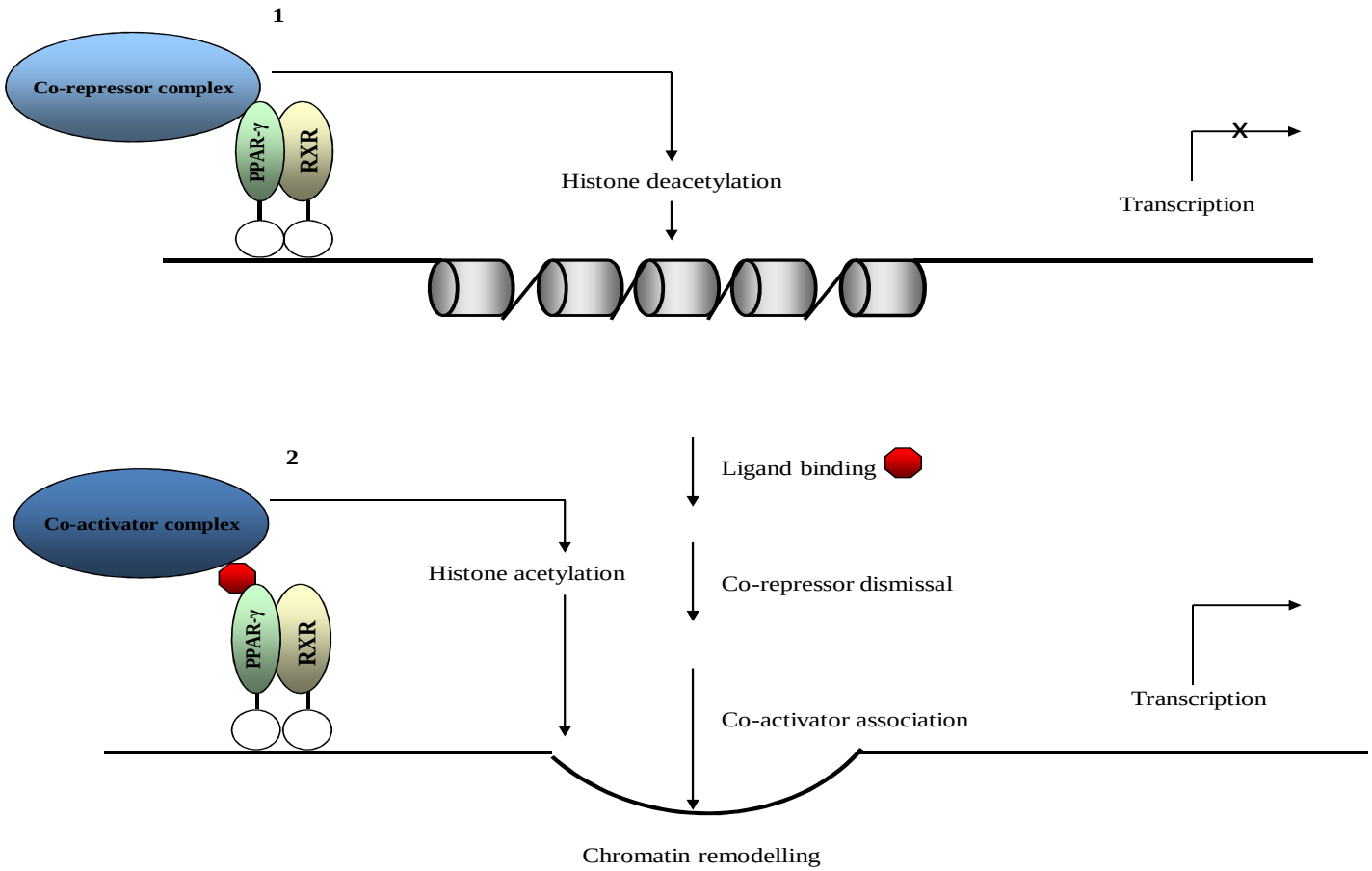


Figure 1.3 - Schematic diagram of the mechanism of PPAR-γ action (adapted from [190])



[1] *Denotes the situation arising in the presence of an unliganded PPAR-γ receptor;* [2] *Denotes the sequence of events set forth following PPAR-γ receptor ligand binding.*

1.4.2 Physiological consequences of PPAR-γ activation

PPAR-γ activation, as evidenced by PPAR-γ mRNA expression, has been shown to play a critical role in adipogenesis and adipocyte differentiation [191, 192]. PPARγ interacts with CCAAT/enhancer-binding protein (-alpha, -beta, -delta), setting a transcriptional network that plays a central role in adipogenesis [191]. This is achieved in a series of steps. Adipogenic hormones, such as insulin and dexamethasone, relay signals to CCAAT/enhancer binding protein-beta and -delta. In turn, CCAAT/enhancer binding protein-beta and -delta synergistically induce the expression of both CCAAT/enhancer binding protein-alpha and PPAR-γ by heterodimerizing with each other [193-196]. CCAAT/enhancer binding protein-alpha and PPAR-γ subsequently enhance each other [197, 198], turning on a battery of genes which are required for the synthesis, uptake and storage of fatty acids and increasing the number of adipocytes [199-201].

Transcription factor PPAR-γ increases insulin sensitivity through a number of mechanisms acting in tandem. PPAR-γ favours the selective expression of genes encoding for proteins involved in fatty acid uptake in adipose tissue, namely adipocyte fatty acid binding protein, acyl-Co A synthase and lipoprotein lipase, without affecting their expression in muscle tissue. This adipocyte free fatty acid 'steal phonomenon' causes a relative depletion of fatty acids in muscle [202, 203]. Moreover, PPAR-γ activation favours the retention of fatty acids in tissues through activation of fatty acid transporters [fatty acid transporter 1 (FATP1) and CD36], phosphoenylpyruvate carboxykinase (PEPCK) and glycerol kinase. PPAR-γ also regulates adipocyte hormone gene expression, enhancing the expression of genes

encoding for insulin sensitizing adipocytokines such as adiponectin, while repressing the expression of genes encoding for adipocytokines implicated in insulin resistance, such as leptin, resistin, tumour necrosis factor-α, 11-β hydroxysteroid dehydrogenase type-1, interleukin-6 and plasminogen activator inhibitor-1. Additionally, PPAR-γ directly enhances adipocyte glucose disposal by inducing glucose transporter-4 (GLUT-4) and c-Cbl associating protein (CAP), the latter being crucial for GLUT4 translocation to the cell surface [204].

1.4.3 Thiazolidinediones and AMPK activation

AMP-activated protein kinase (AMPK), a highly conserved major regulator of cellular and whole-body energy homeostasis, is also a target of thiazolidinedione action [89]. Thiazolidinediones are reported to activate AMPK via two independent mechanisms. Like the biguanides, thiazolidinediones appear to exert their acute effects on AMPK by inhibiting complex 1 of the respiratory chain [205], therebye explaining the associated drug-induced increase in the cellular AMP:ATP ratio [206, 207]. Morerover, as outlined above, thiazolidinedione-induced PPAR-γ activation induces the expression and release of adiponectin from human and rodent adipocytes [208]. Adiponectin in turn activates AMPK in the liver and skeletal muscle, reducing hepatic gluconeogenesis, and favouring glucose uptake and fatty acid oxidation [209]. Mice lacking adiponectin fail to exhibit thiazolidinedone-induced AMPK activation and improvements in glucose tolerance [210].

In conclusion, metformin and thiazolidinediones improve insulin sensitivity through multifaceted but complementary approaches: both act as AMPK activators, but

metformin predominantly targets hepatic glucose output while thiazolidinediones regulate peripheral glucose and fatty acid uptake predominantly in adipose tissue via PPAR-γ receptor modulation. In both cases, glucose control is improved with a minimal risk of hypoglycaemia.

Section II - Heart failure in diabetes, with particular reference to thiazolidinedione therapy

1.5 Concurrence of diabetes and heart failure

In recent years, the relationship between HF and diabetes has been increasingly recognised and investigated. The American Heart Association classifies diabetes as a high risk factor for the development of HF [211]. There is evidence for diabetes related effects on HF prevalence, incidence and mortality. It is to be noted that large diabetes trials either excluded patients with HF [e.g. UKPDS [212], Non-Insulin Dependent Diabetes, Hypertension, Microalbuminuria or Proteinuria, Cardiovascular Events, and Ramipril (DIABHYCAR) [213], DCCT [214]] or did not report HF as a co-morbidity [e.g. Collaborative Atorvastatin Diabetes Study (CARDS) [215], Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) [216], PROspective pioglitAzone Clinical Trial in macroVascular Events (PROactive) [217]]. As for the occurrence of diabetes in HF, figures need to be interpreted with caution, given that the strict recruitment criteria for the individual trials exclude individuals at higher risk of diabetes (such as older age groups, and renal dysfunction). Similarly, it is difficult to extract population-based estimates of the incidence of HF in diabetes from large trials such as the UKPDS, which solely recruited patients with newly diagnosed diabetes (mean age = 53 years). Given these constraints, prevalence and incidence data have been retrieved largely from population based studies.

1.5.1 Prevalence

Prevalence of heart failure in diabetes: The prevalence of HF in diabetes stands at 12% [218], increasing to 22% among individuals aged above 64 years [219], compared to 1-4% in the general population [218].

Prevalence of diabetes in heart failure: Diabetes was reported as being four times more prevalent among patients with newly diagnosed HF [220]. Diabetes occurs in 12-30% of individuals with symptomatic HF [218, 220-222], and in 33-40% of hospital admissions resulting from HF [223-225]. A retrospective analysis of around 45,000 patients with idiopathic cardiomyopathy confimed similar results, namely significantly higher prevalence rates among the diabetic sub-population [26.6% vs 17.2%, corresponding to a relative odds of 1.58 (95% CI 1.55, 1.62) after adjusting for age, sex, hypertension and median income [226]]. Data from other smaller epidemiological studies of patients with left ventricular systolic dysfunction (ranging from 188 to 3960 patients) reported diabetes prevalence rates of 6-25.5%, although there were considerable differences in patient age and in the definition of left ventricular systolic dysfunction between studies [227-233]. It is as yet unclear whether the prevalence of diabetes in HF varies according to ethnic group [224, 225].

1.5.2 Incidence

Incidence of heart failure in diabetes: Diabetes has also been identified as a major contributor to the incidence of this cardiac condition. A diagnosis of HF was 2.4

times as likely among diabetic men and 5.1 times as likely among diabetic women who participated in the Framingham Heart Study (age range 45-74 years). This association was independent of age, obesity, hypertension, dyslipidaemia and coronary artery disease. The effect was even more pronounced in individuals younger than 65 years, where the risk of developing HF was estimated at 4 fold and 8 fold higher for diabetic men and women respectively [234]. The National Health and Nutrition Examination Survey (NHANES) [235] and Cardiovascular Health Study [236] reported hazard ratios of 1.85 (95% CI 1.51, 2.28) and 1.74 (95% CI 1.38, 2.19) respectively for HF development in diabetic patients. In Iceland, the age-adjusted odds ratio for the development of HF was 2.8 (95% CI 2.2, 3.6) in diabetic patients, compared to their non-diabetic counterparts [218].

A cross-sectional study comparing the incidence of HF between diabetic and non-diabetic subgroups of 2737 American elderly patients (mean age 81 ± 9 years) revealed that HF developed in 39% of diabetic patients compared with 23% of non-diabetic individuals ($p < 0.0001$). Relative risk was estimated at 1.3 for the diabetic population [237]. A large US cohort study of 115,803 diabetes patients over 64 years of age reported 126 cases of incident HF per 1000 patient years [219].

The United Kingdom Diabetes Prospective Diabetes Study (UKPDS) reported that the risk of HF increased with worsening glycaemic control in T2DM patients, such that there was a 16% reduction in the risk of HF for every 1% reduction in glycosylated haemoglobin (HbA1c) [212]. Conversely, each 1% increase in HbA1c was linked to an 8% increase in HF risk (95% CI 5, 12%) in a US study [238]. A 2.5 unit increase in BMI has been associated with a 12% increase in the risk of HF in

diabetic patients [239]. A similar relationship was reported in another study [240]. Increasing age [213, 219, 239], use of insulin [239], and duration of diabetes [239] have also been identified as risk factors.

Coronary heart disease is a risk factor for HF in diabetes [219, 239, 240]. Morerover, diabetic patients are more likely to develop HF following a myocardial infarction despite comparable infarct sizes [241]. Diabetic patients with retinopathy have also been recognised as being at an increased risk of HF [242]. Subgroup analysis of the Multi-Ethnic Study of Atherosclerosis (MESA) cohort showed broadly similar findings, namely an association between retinal arteriolar narrowing and left ventricular remodelling [243]. Other studies reported proteinuria and albuminuria [213, 240, 244], nephropathy [219] and end-stage renal disease [219, 239] as additional risk factors for HF in diabetes. Overall, these results support the concept of microvascular aetiology for HF in diabetes, or an interaction between large and small vessel disease.

Incidence of diabetes in heart failure: Only one non-clinical trial population study investigated the development of diabetes among patients diagnosed with HF. The 3 year incidence of diabetes was 28.8% in elderly Italian patients with HF compared with 18.3% in individuals without HF [220].

1.6 Mortality risks associated with heart failure

Diabetes and mortality in patients with heart failure: Diabetes is a recognised independent risk factor of death among patients with established HF. This is borne

out of the results of a number of studies. However, it is unclear whether this risk holds only for individuals in whom HF is caused by a specific aetiology. Analysis of clinical trial population data from the Studies of Left Ventricular Dysfunction (SOLVD) [245, 246], Beta-blocker Evaluation in Survival Trial (BEST) [247] and Digitalis Investigation Group (DIG) [248] studies suggested that mortality risk was confined to individuals with HF of ischaemic aetiology, in contrast to a US community cohort based study, which reported an association with non-ischaemic HF [222]. In contrast, diabetes posed a mortality risk to HF patients of either category in the Danish Investigations of Arrhythmia and Mortality ON Dofetilide Heart Failure (DIAMOND-HF) [249] and Candesartan in Heart failure Assessment of Reduction in Mortality and morbidity (CHARM) [250] clinical trials. Differences in study outcomes may be borne out of underdiagnosis of coronary artery disease in diabetes and differences in study population characteristics and study design. The hazard ratios for death from pump failure in diabetic individuals were reported as 1.44 (95% CI 1.18, 1.76) and 1.50 (95% CI 1.15, 1.74) in the SOLVD [245, 246] and BEST [247] trials respectively. Subgroup analysis of data from the Framingham study suggested that the risk of diabetes related mortality was confined to female HF patients [251]. The results may have been influenced by the small sample size, and may explain why such gender differences were not confirmed in other studies.

Interestingly, a low HbA1c has been identified as a mortality risk factor for HF in diabetic patients in one observational study [252]. Analysing for 123 individuals with advanced HF, 2 year all-cause mortality rates were significantly higher for patients with an HbA1c of 7 or less compared to those with higher values (35% vs

20%). These figures need to be interpreted with caution, and probably reflect the effects of cachexia, which is inherent to individuals with advanced HF.

Heart failure and mortality in patients with diabetes: Current evidence suggests that diabetes patients who develop HF are at an increased risk of mortality. The DIABHYCAR study showed that T2DM patients who develop HF had a twelve-fold higher annual mortality rate compared to diabetic individuals who were not diagnosed with HF (36.4% vs 3.2%). This study was carried out in individuals above 50 years of age and urinary albumin concentrations equalling or exceeding 20mg/L [213]. A large US population study recruiting data from diabetic patients aged 65 years or older reported a five year survival of 12.5% for individuals who developed HF, as compared to 80% for those who did not develop this cardiovascular condition [219].

1.7 Thiazolidinediones and oedema

A meta-analysis of 26 prospective, randomised, placebo-controlled or comparative studies investigating the incidence of oedema in thiazolidinedione-treated patients concluded that the latter are associated with a doubling of risk [pooled OR 2.26 (95% CI 2.02, 2.53); $p < 0.00001$]. Oedema rates were approximately three fold higher for rosiglitazone-treated patients [pooled OR 2.74 (95% CI 2.33, 3.14)]. Open labelled studies reported a higher thiazolidinedione-associated risk [pooled OR 6.74 (95% CI 3.32, 13.71); $p < 0.00001$] [253]. However, recruited studies adopted different definitions of oedema. Moreover, only two studies used objective methods to evaluate this adverse effect, while severity was only reported in three studies.

Available data did not permit investigating whether concomitant drugs mitigate or exacerbate the risk of fluid overload [253].

1.8 Thiazolidinediones and heart failure

The clinical benefits and widespread use of thiazolidinediones have been hampered by concerns on their cardiovascular safety profile, namely ischaemic heart disease (rosiglitazone) and an association with fluid retention/HF. Early clinical efficacy/safety (phase II) trials had failed to clearly demonstrate any relationship between pioglotazone or rosiglitazone monotherapy and the development of HF, although the risk may be increased when the drug is used in combination with insulin. On the other hand, four major prospective randomized trials and recent meta-analyses of data from these and other studies have attested this relationship.

1.8.1 Clinical efficacy/safety trials

The package inserts for rosiglitazone maleate (Avandia®) [254] and pioglotazone hydrochloride (Actos®) [255] yield useful prescribing advice in this regard. Both drugs are deemed contraindicated in individuals with New York Heart Association (NYHA) HF classes III and IV. Additionally, the manufacturers do not recommend their use in individuals with symptomatic HF. Individuals with NYHA HF classes I and II are deemed as being at an increased risk of 'other cardiovascular effects' when treated with Avandia®. The manufacturers of Actos® recommend that this drug should be commenced at the lowest approved dose if contemplated for use in T2DM patients with NYHA HF class II. Any further dose escalation, if necessary, should be

carried out after ‘several months of treatment’ and ‘careful monitoring for weight gain, oedema, or signs and symptoms of CHF exacerbation’. When evaluating available data, one must keep in mind that individuals with NYHA HF class III and IV were not included in the pre-approval clinical trials.

(i) Unpublished clinical safety trials for pioglitazone hydrochloride (Actos®)

In their package insert [255], the manufacturers of pioglitazone hydrochloride refer to a double-blind placebo controlled pre-approval clinical trial involving 566 insulin-treated T2DM patients followed up for 16 weeks. Participants were randomised to pioglitazone at 15mg or 30 mg daily, or placebo, and included individuals with arterial hypertension (57.2%), coronary heart disease (19.6%), history of MI (8.8%), history of angina pectoris (4.4%), congestive heart failure (2.3%) and stroke and/or transient ischemic attack (4.1%). 2 patients on pioglitazone 15mg and 2 of those on pioglitazone 30mg developed CHF. Although this adverse event was not reported in placebo-treated individuals, it was restricted to individuals with a past history of cardiovascular disease.

A 24 week post-marketing study compared the safety profile of pioglitazone (n = 262) and glyburide (n = 256) in uncontrolled T2DM patients (mean baseline HbA1c 8.8%) characterised by NYHA class III and IV HF and a baseline ejection fraction less than 40% (mean 30%). Overnight hospitalization for HF was increased, reported in 9.9% of pioglitazone-treated patients compared to 4.7% of those managed with glyburide. Treatment differences were first noted after 6 weeks of therapy.

Pioglitazone-associated hospitalization for HF was more common in individuals aged over 64 years and those treated with insulin at baseline.

Statistical analyses of the differences between treatment groups are not reported for either of the two studies, which are not referenced in the package insert.

(ii) Unpublished clinical safety trials for rosiglitazone maleate (Avandia®)

The package insert for rosiglitazone maleate [254] refers to a 52 week double-blind placebo-controlled study carried out in 224 T2DM patients with NYHA class I or II HF and a baseline ejection fraction equalling or less than 45%, treated with background antidiabetic and CHF therapy. While the investigators reported no differences in change in ejection fraction between treatment groups, rosiglitazone-treated patients were more prone to adverse cardiovascular events (new or worsening oedema, new or worsening dyspnoea, increases in CHF medication, cardiovascular hospitalization, cardiovascular deaths) compared to their placebo-treated counterparts. It is not clear whether this study was carried out in the pre-approval phase, and statistical analysis of the differences between treatment groups is not reported. This study is not referenced in the package insert.

1.8.2 Prospective randomized trials

Four large-scale randomized prospective trials (tables 1.2 and 1.3) cumulatively recruiting over nineteen thousand patients have yielded valuable information on the safety profile of thiazolidinediones. They recruited individuals from four very

different populations: patients with pre-diabetes (impaired glucose tolerance or impaired fasting glucose) and no evidence of cardiovascular disease [Diabetes REduction Assessment with ramipril and rosiglitazone Medication (DREAM)] [152], pharmacologically naïve T2DM patients [A Diabetes Outcome Progression Trial (ADOPT)] [154], T2DM patients (some with previous cardiovascular disease) inadequately controlled on a sulphonylurea or metformin [Rosiglitazone Evaluate for Cardiovascular outcomes in ORal agent combination therapy for type 2 Diabetes (RECORD)] [153], and high risk T2DM patients with established cardiovascular disease [PROspective pioglitAzone Clinical Trial in macroVascular Events (PROactive)] [256]. Two of these trials compared thiazolidinedione treatment with placebo therapy (DREAM, PROactive) [152, 256], while the other two trials (ADOPT, RECORD) [153, 154] compared thiazolidinedione therapy with metformin and sulphonylureas. Three studies (DREAM, ADOPT, RECORD) randomised patients to thiazolidinedone treatment with rosiglitazone [152-154], while the PROactive study randomised individuals to pioglitazone [256]. The mean age of the patients at recruitment ranged from 54.7 to 61.8 years. Baseline HbA1c was sub-optimal, ranging from 7.4-7.9 in three [153, 154, 256] out of four [152-154, 256] trials (it was not reported in the DREAM trial which recruited patients with pre-diabetes [152]). The PROactive trial excluded patients with NYHA HF class II or above [256]. The DREAM [152], ADOPT [154] and RECORD [153] studies excluded any individual with HF at recruitment.

All four trials reported a significant excess of thiazolidinedione-treated patients with HF. The DREAM trial [152] defined HF as acute treatment with at least two of the following criteria: typical signs and symptoms, typical radiological evidence and the

use of diuretics, vasodilators or inotropes. 14 rosiglitazone-treated patients and 2 placebo-treated patients developed HF during the study [HR 7.03 (95% CI 1.60, 30.9); p = 0.01). There were no reports of deaths from HF during the study, although the investigators reported a death from myocardial infarction in one rosiglitazone-treated patient who had developed HF. Additionally, 174 (6.8%) of the 2547 rosiglitazone-treated patients had developed peripheral oedema by the final visit, compared to 124 (4.9%) of the 2554 patients randomised to a placebo (p = 0.003). The authors also reported a significant mean body weight increase of 2.2 kg in the rosiglitazone-treated group compared to placebo (p < 0.0001). While rosiglitazone therapy significantly reduced the composite endpoint of incident diabetes or death (p < 0.0001), there were no significant differences between treatment groups in composite cardiovascular endpoints (comprising myocardial infarction, stroke, cardiovascular death, revascularization procedure, HF, new angina with objective evidence of ischaemia, ventricular arrhythmias requiring resuscitation), overall mortality, myocardial infarction, new angina or stroke between the treatment groups. The study did not report information on differences in changes in lipid profile between rosiglitazone and placebo-treated groups.

The ADOPT trial [154] sought to investigate differences in outcomes between T2DM patients randomised to monotherapy with rosiglitazone (n = 1456), metformin (n = 1454) or glyburide (n = 1441). Although the study protocol excluded patients with known CHF, retrospective analysis of source data identified this diagnosis in 17 study patients at recruitment (5 in the rosiglitazone group, 6 in the metformin group and 6 in the glyburide group). Only 1 of these patients, randomised to metformin, subsequently developed a HF event during the study. There were no

significant differences in the number of patients with HF between the rosiglitazone and metformin-treated groups at the end of the study [22 vs 19; HR 1.22 (95% CI 0.66, 2.26); p = 0.52). Although a greater number of rosiglitazone-treated patients developed HF compared to those randomised to glyburide (22 vs 9), the difference achieved only borderline statistical significance [HR 2.20 (95% CI 1.01, 4.79); p = 0.05]. Serious HF events (defined as life threatening, fatal, disabling, requiring hospitalization or prolongation of hospital stay, associated with a congenital anomaly, cancer or a drug overdose, regarded as such by the investigator or suggesting substantial hazard, contraindication, side-effect or precaution) affected 12 patients in the rosiglitazone-treated group, 12 patients in the metformin-treated group and 3 glyburide-treated individuals ($p < 0.05$ for the comparison between rosiglitazone and glyburide-treated patients). A significantly greater number of rosiglitazone-treated patients developed peripheral oedema compared to those on metformin (205 vs 104; $p < 0.001$) or glyburide (205 vs 123, $p < 0.001$). Rosiglitazone-treated patients gained 4.8kg (95% CI 4.3, 5.3) in weight compared to a reduction of 2.9 kg (95% CI -3.4, -2.3) for metformin-treated patients and an increase of 1.6kg (95% CI 1.0, 2.2) for glyburide-treated individuals. At the end of the study, rosiglitazone-treated patients were 6.9 kg heavier (95% CI 6.3, 7.4) than their metformin-treated counterparts ($p < 0.001$) and 2.5 kg (95% CI 2.0, 3.1) heavier than patients randomised to glyburide ($p < 0.001$). The study confirmed that thiazolidinedione treatment is associated with a lower rate of monotherapy failure at 5 years (defined as fasting plasma glucose exceeding 10 mmol/L) compared to metformin or glyburide ($p < 0.001$ for both comparisons). Rosiglitazone-treated patients achieved significantly greater reductions in their glycated haemoglobin level compared to those randomized to metformin [reduction difference of 0.13% (95% CI

-0.22, -0.05); p = 0.002] or glyburide [reduction difference of 0.42% (95% CI -0.50, -0.33); p < 0.001]. Despite rosiglitazone being associated with higher LDL cholesterol levels at the end of the study, compared to metformin [2.69 (95% CI 2.63, 2.75) vs 2.50 (95% CI 2.44, 2.55) mmol/L; p < 0.001] and glyburide [2.69 (95% CI 2.63, 2.75) vs 2.57 (95% CI 2.51, 2.64); p = 0.008], this did not translate into any significant differences in the number of patients with fatal or nonfatal MI, stroke or overall mortality between the treatment groups.

The conclusions borne out of the ADOPT study have been the subject of considerable debate. Although the study yielded useful data concerning drug associated changes in body weight, oedema and HF, it was primarily designed to compare durability of glycaemic control between three treatment groups. Given that the investigators only reported outcomes at the end of the study period (48 months for metformin and rosiglitazone-treated patients, 39.6 months for glyburide-treated patients), it is not possible to compare outcomes after 1, 2 or 3 years. Morerover, high dropout rates were reported for the three treatment groups (63% for rosiglitazone, 62.1% for metformin, 56% for glyburide), potentially introducing hidden biases in reported adverse event rates [257]. Complications such as weight gain would be expected to adversely affect drug compliance.

The RECORD trial [153] comprised an unblinded prospective study recruiting T2DM patients inadequately controlled on metformin or sulphonylurea monotherapy. The investigators compared primary and secondary cardiovascular prevention between patients randomised to treatment with rosiglitazone or metformin-sulphonylurea combination. By the end of the trial, a significantly greater

number of rosiglitazone-treated patients had developed new-onset HF leading to hospitalization (undefined in the study) or death compared to their comparator-treated counterparts [61 vs 29; HR 2.1 (95% CI 1.35, 3.27); $p < 0.001$]. There were 10 deaths attributed to HF in the rosiglitazone-treated group and 2 in the sulphonylurea/metformin group; these figures were not compared statistically. However there was no significant difference in all cause mortality between the treatment groups [136 (rosiglitazone) vs 157 (comparator)], as the higher mortality from HF was offset by a lower occurrence of death from stroke [0 (rosiglitazone) vs 5 (comparator)], myocardial infarction [7 (rosiglitazone) vs 10 (comparator)] and other cardiovascular causes [43 (rosiglitazone) vs 54 (comparator)]. The authors did not report any significant differences in the occurrence of myocardial infarction [64 (rosiglitazone) vs 56 (comparator)] and stroke [46 (rosiglitazone) vs 63 (comparator)] between the treatment groups. The authors maintained that the excessive mortality from HF for rosiglitazone-treated patients was compatible with the increased occurrence of HF seen in this treatment group, and that the excess relative risk of HF for these patients was similar for individuals with and without ischaemic heart disease. Metformin-treated patients randomised to additional treatment with rosiglitazone gained more weight compared those treated with adjunct sulphonylurea (+3.8 vs 0.0 kg; $p < 0.0001$). Sulphonylurea-treated patients randomized to adjunct rosiglitazone gained more weight than those randomised to additional treatment with metformin (+4.1 vs -1.5 kg; $p < 0.0001$).

A follow-up paper focussing on occurrence of HF events in RECORD [258] reported that the mean duration (± SD) of admission for HF in the rosiglitazone group [69 events, 10.5 (± 6.6) days] was similar to that for the active control group [36 events,

9.6 (± 5.3) days]. Despite more incident HF events in the rosiglitazone group than in the active control group [61 (rosiglitazone) vs 29 (active control); HR 2.10 (95% CI 1.35, 3.27); $p < 0.001$], recurrent HF events were similar in both treatment groups [12 (rosiglitazone) vs 6 (active control)]. The estimated excess event rate for HF was 2.6 (95% CI 1.1, 4.1) per 1000 person-years. Of the ten deaths complicating HF in the rosiglitazone group, four were incident HF events while six deaths occurred following a recurrent HF episode. There were no fatal incident HF events in the control group, while two deaths complicated a recurrent HF episode. 17 (30%) of the 57 rosiglitazone-treated patients who survived a first HF event subsequently died, compared with 8 (28%) of patients in the active control group. Thiazolidinedone treatment was associated with a similar relative risk increase but a doubled absolute risk for HF events in patients with a history of ischaemic heart disease (IHD) compared with their IHD free counterparts [4.4% of rosiglitazone-treated patients with prior IHD vs 2.4% of rosiglitazone-treated patients without prior IHD; RR 2.16 (95% CI 0.94, 4.94) for patients with prior IHD vs RR 2.10 (95% CI 1.25, 3.51) for patients without prior IHD]. Rosiglitazone assignment [HR 2.34 (95% CI 1.47, 3.72) vs control], age [1.10 (95% CI 1.07, 1.13) per one-year increase], BMI [HR 1.11 (95% CI 1.06, 1.15) per 1kg/m^2 increase], systolic blood pressure at baseline [HR 2.74 (95% CI 1.40, 5.36) for baseline antihypertensive therapy vs no therapy; HR 1.66 (95% CI 1.06, 2.62) for uncontrolled hypertension vs no uncontrolled hypertension] and urinary albumin:creatinine ratio [HR 2.95 (95% CI 1.90, 2.47) for microalbuminuria/proteinuria vs normoalbuminuria] were independent predictors of HF events. A history of previous cardiovascular disease, gender and duration of diabetes were not predictive of HF in this cohort [258].

Although RECORD remains the only large, randomised, long-term trial assessing the cardiovascular safety of rosiglitazone compared to other glucose lowering agents in T2DM, its results have been questioned on account of certain built-in limitations, namely its open-labelled design, its relatively small size (for a cardiovascular trial) and the choice of primary endpoint. Importantly, the provision for investigator option in referring potential events for adjudication and the publication of an unplanned interim analysis of its results [259] triggered by the publication of meta-analyses questioning the cardiovascular safety of thiazolidinediones may have inherently biased the cardiovascular outcome results of RECORD. These observations led the FDA to request a re-analysis of RECORD data in a bid to clarify these conflicting conclusions [158]. Including an additional 328 patient-years of follow-up, RECORD investigators confirmed initial findings [revised HR for rosiglitazone vs metformin/sulphonylurea for the composite endpoint of death (cardiovascular/unknown cause), myocardial infarction or stroke being 0.95 (95% CI 0.78, 1.17) vs 0.93 (95% CI 0.74, 1.15) in the original analysis; revised HR for myocardial infarction 1.13 (95% CI 0.80, 1.59) vs 1.14 (0.80, 1.63); revised HR for stroke 0.79 (95% CI 0.54, 1.14) vs 0.72 (95% CI 0.49, 1.06); unchanged for all-cause death]. This re-analysis made no reference to HF events or oedema [260].

The PROactive study [256] randomized high risk T2DM patients with a background of macrovascular disease to additional treatment with pioglitazone or placebo for a mean duration of 34.5 months. Despite an unfavourable effect on LDL cholesterol [+7.2% over baseline (pioglitazone) vs +4.9% over baseline (placebo); p = 0.003], pioglitazone was shown to reduce the composite endpoint of all-cause mortality, non-fatal myocardial infarction and stroke in high risk T2DM patients [301

(pioglitazone) vs 358 (placebo) HR 0.84 (95% CI 0.72, 0.98); p = 0.02] [256]. The investigators reported that a HF event (defined as evidence of ventricular dysfunction e.g. electrocardiogram (ECG), echocardiogram or auscultation, accompanied by signs or symptoms of HF) occurred in 10.8% of pioglitazone-treated patients compared with 7.5% of those randomized to a placebo (p<0.0001). Although pioglitazone therapy was associated with a significantly increased risk of a serious HF event, (defined as HF leading to or prolonging a hospitalisation stay) [149 (5.7%) (pioglitazone) vs 108 (4.1%) (placebo); HR 1.41 (95% CI 1.10, 1.80); p = 0.007], mortality rates from HF were comparable to placebo-treated patients [25 (0.96%) (pioglitazone) vs 22 (0.84%) (placebo); HR 1.15 (95% CI 0.65, 2.03); p = 0.639] [256]. Further analyzing data from patients with a serious HF event, a follow-up paper reported that subsequent all-cause mortality was proportionately lower with pioglitazone, although the difference did not reach statistical significance [40 (26.8%) (pioglitazone) vs 37 (34.3%) (placebo); HR 0.71 (95% CI 0.454, 1.111); p = 0.1338] [261]. Significantly fewer such patients subsequently developed an event in the secondary endpoint, comprising a composite of all-cause mortality, non-fatal myocardial infarction and stroke [52 of 149 (34.9%) (pioglitazone) vs 51 of 108 (47.2%) (placebo); HR 0.64 (95% CI 0.436, 0.946); p = 0.025] [261]. Although fewer pioglitazone-treated patients who had developed a serious HF event went on to develop an event in the primary endpoint (composite of all-cause mortality, non-fatal myocardial infarction [including silent myocardial infarction], stroke, acute coronary syndrome, endovascular or surgical intervention in the coronary or leg arteries, and amputation above the ankle), the difference did not reach statistical significance [71 of 149 (47.7%) (pioglitazone) vs 62 of 108 (57.4%) (placebo); HR 0.72 (95% CI 0.512, 1.013); p = 0.0593] [261]. Analyzing data from individuals who developed

serious HF, there were no significant differences between the treatment groups in the median number of days spent in hospital (11 days in each treatment group) and in the median number of days spent in intensive care/high dependency unit [4 days (pioglitazone) vs 3 (placebo); p = 0.584] [261]. Most serious HF events resolved in either group [77.9% (pioglitazone) vs 74.1% (placebo); p = 0.4822]. 22.8% of pioglitazone-treated patients and 15.7% of placebo-treated patients had a serious HF event that resulted in discontinuation from the study; this difference did not reach statistical significance (p = 0.1602) [261]. Significant predictors of a serious HF event on multivariate analysis were randomisation to pioglitazone [HR 1.53 (95% CI 1.183, 1.979)], age in years [HR 1.07 (95% CI 1.044, 1.087)], BMI [HR 1.03 (95% CI 1.007, 1.061)], HbA1c of/exceeding 7.5% [HR 1.43 (95% CI 1.078, 1.895)], diabetes duration of/exceeding 10 years vs less than 5 years [HR 1.53 (95% CI 1.107, 2.115)], creatinine > 130 μmol/L [HR 2.7 (95% CI 1.796, 4.061)], diuretic use [HR 2.10 (95% CI 1.62, 2.732)], LDL cholesterol > 4 mmol/L vs < 3mmol/L [HR 1.74 (95% CI 1.245, 2.442)], and previous myocardial infarction [HR 1.70 (95% CI 1.317, 2.205)] [261]. Despite its usefulness, HF data from the PROactive trial need to be interpreted with caution given the occurrence of potentially confounding baseline differences between pioglitazone and placebo patients who developed serious HF, namely higher baseline prevalence rates for percutaneous coronary intervention/coronary artery bypass graft and transient ischaemic attacks. Such patients were also characterized by a higher baseline systolic blood pressure (data not shown) [261] – the latter having been reported as a predictor of HF events complicating rosiglitazone therapy in the RECORD trial [262] Moreover, a higher proportion of pioglitazone-treated patients who went on to develop serious HF had been receiving nonsteroidal anti-inflammatory drugs [12% (pioglitazone) vs 1%

(placebo)] and loop diuretics [40% (pioglitazone) vs 30% (placebo)] at baseline, albeit the reverse was true for baseline insulin therapy [36% (pioglitazone) vs 44% (placebo)] [261]. Pioglitazone therapy was associated with significantly higher risk for a non-serious HF event [6.4% (pioglitazone) vs 4.3% (placebo); $p = 0.0007$], although a similar proportion of such patients progressed to a serious HF event [21 (pioglitazone) vs 20 (placebo)]. In keeping with the results of other studies, Erdmann et al. reported significant differences in change in weight between the treatment groups at the end of the study [+3.6 kg (pioglitazone) vs -0.4 kg (placebo); $p<0.0001$]. Peripheral oedema occurring in the absence of HF occurred more commonly in pioglitazone-treated patients [563 (21.6%) (pioglitazone) vs 341 (13.0%) (placebo); $p < 0.0001$] [261]. Oedema was more likely to precede a serious HF event in pioglitazone-treated patients [51 out of 149 (34.2%) (pioglitazone) vs 26 out of 108 (24.1%) (placebo)]; this difference was not statistically compared between allocation groups [261].

Table 1.2 - The four major prospective thiazolidinedione trials: study design and baseline characteristics of participants

Study	*Year*	*Design*	*Blinding of investigator (patients)*	*Diabetes status and treatment*	*Baseline macrovascular disease (%)*	*Number of patients randomised (completed)*	*TZD (daily dose in mg)*	*Comparator (daily dose in mg)*	*Duration in months (or as stated)*	*Mean age at recruitment (years)*	*Baseline anthropometry*	*Baseline HbA_{1c} (%)*
DREAM	2006	Prospective randomized Intention to treat analysis	Yes (Yes)	Pre-diabetes (IFG or IGT)	No evidence	Rosi: 2635 (1863) PL: 2634 (1976)	Rosi (8) forced titration	PL	36	54.7	*Wt* 84.9 kg *BMI* 30.9 kg/m^2	a
ADOPT	2006	Prospective randomized Intention to treat analyses	Yes (Yes)	Pharmacologically naive T2DM	b ¶	Rosi 1456 (917) MTF 1454 (903) Glyb 1441 (807)	Rosi (4-8)	MTF (500-2000) or glyb (2.5-7.5)	Rosi:48 MTF:48 Glyb: 39.6	56.9	*Wt* 91.7kg *BMI* 32.2kg/m^2	7.4
RECORD	2009	Prospective randomized Intention to treat analysis	No (No)	T2DM inadequately controlled with SU or MF monotherapy	IHD: 17.4 Stroke: 2.4 TIA: 2.2 PAD: 4.9	Rosi: 2220 (1835) Comp: 2227 (1798)	Rosi (4-8)	MTF (2550) or Glib (15) /glic(240)/glim (4)	66	58.4	*Wt* 89.0 kg	7.9
PROactive	2005	Prospective Randomized Intention to treat analysis	Yes (Yes)	T2DM treated with diet or OHAs or insulin	All patients	Pio: 2605 (2427) PL: 2633 (2446)	Pio (15-45)	PL	34.5	61.8	*BMI* 30.9 kg/m^2	7.9

Table 1.3 - The four major prospective thiazolidinedione trials: study outcomes

Study	*Year*	*Primary endpoint*	*Effect on primary endpoint*	*Vascular secondary endpoint(s)*	*Effect on secondary endpoint*	*Effect on HF*	*Effect on HF mortality*	*Peripheral oedema*	*Effect on weight (kg)*
DREAM	2006	Composite of incident diabetes or death	306 (Rosi) vs 686 (PL) § HR 0.40 (0.35-0.46) (p<0.0001)	Composite CVS events (MI, stroke, CVS death, revasc proc, HF, new angina with objective ischaemia evidence, vent arrhythmia requiring resusc	75 (Rosi) vs 55 (PL) § HR 1.37 (0.97-1.94) (p= 0.08)	14 (Rosi) vs 2 (PL) HR 7.03 (1.60-30.9) (p=0.01)	None reported	174 (Rosi) vs 124 (PL) § (p = 0.003)	Rosi increased wt by 2.2kg compared to PL (p<0.0001)
ADOPT	2006	Monotherapy failure at 5 years (FPG>10 mmol/L)	15% (Rosi) vs 21% (MTF) § (p<0.001); 15% (Rosi) vs 63% (Glyb) § (p<0.001)	c	c	22 (Rosi) vs 19 (MTF) § HR 1.22 (0.66-1.26) (p = 0.52) ; 22 (Rosi) vs 9 (Glyb) § HR 2.20 (1.01-4.79) (p=0.05)		205 (Rosi) vs 104 (MTF) § (p < 0.001); 205 (Rosi) vs 123 (Glyb)§ (p<0.001)	*Rosi vs MTF* 6.9 (6.3-7.4) (p<0.001) *Rosi vs Glyb* 2.5 (2.0-3.1) (p<0.001)
RECORD	2009	Cardiovascular hospitalisation or cardiovascular death	321 (Rosi) vs 323 (Comp) § HR 0.99 (0.85-1.16) (p = 0.93)	Composite of cardiovascular death, MI and stroke	154 (Rosi) vs 165 (Comp) § HR 0.93 (0.74-1.15) (p = 0.50)	61 (Rosi) vs 29 (Comp) § HR 2.1 (1.35-3.27) (p= 0.001)	*All cause* 136 (Rosi) vs 157 (Comp) § (p = 0.19) *Fatal HF* 10 (Rosi) vs 2 (Comp) b	a	*Background MTF* +3.8 (Rosi) vs 0.0 (SU) (p<0.0001) *Background SU* +4.1 (Rosi) vs -1.5 (MTF) (p<0.0001)
PROactive	2005	Composite of all-cause mortality, non-fatal MI, ACS, stroke, leg/coronary endovascular/surgical intervention, above ankle amputation	514 (Pio) vs 572 (PL) § HR 0.90 (0.8-1.02) (p = 0.095)	Composite of all-cause mortality, non-fatal MI (excluding silent MI) or stroke	301 (Pio) vs 358 (PL) § HR 0.84 (0.72-0.98) (p = 0.027)	281 (Pio) vs 198 (PL) § (p<0.0001) *Admissions* 149 (Pio) vs 108 (PL) § (p = 0.007)	*Fatal HF* 25 (Pio) vs 22 (PL) § (p=0.634)	*In the absence of HF* 562 (Pio) vs 341 (PL) § b	+3.6 (Pio) vs -0.4 (PL) (p<0.0001)

Table 1.3 continued - The four major prospective thiazolidinedione trials - study outcomes.

Author	*Year*	*Effect on IHD*	*Effect on IHD mortality*	*Effect on stroke*	*Effect on stroke mortality*	*Overall effect on mortality*	*Effect on HbA1c*	*Effect on LDL (mmol/L)*
DREAM	2006	*MI* 15 (Rosi) vs 9 (PL) § HR 1.66 (0.73-3.80) (p-0.2) *New angina* 24(Rosi) vs 20 (PL) § HR 1.20 (0.66-2.17)(p=0.5)	a	7 (Rosi) vs 5 (PL) § HR 1.39 (0.44-4.40) (p=0.6)	a	30 (Rosi) vs 33 (PL) § HR 0.91 (0.55-1.49) (p = 0.7)	a	a
ADOPT	2006	*Nonfatal MI* 25 (Rosi) vs 21(MTF) § (p = NS) 25(Rosi) vs 15 (Glyb) § (p = NS)	*Fatal MI* 2 (Rosi) vs 2 (MTF) § (p = NS) 2 (Rosi) vs 3 (Glyb) § (p = NS)	16 (Rosi) vs 19 (MTF) § (p= NS) 16 (Rosi) vs 17 (Glyb) § (p= NS)	a	34 (Rosi) vs 31 (MTF) § (p = NS) 34 (Rosi) vs 31 (Glyb) § (p = NS)	Rosi vs MTF -0.13 (-0.22 to -0.05) (p = 0.002); Rosi vs Glyb -0.42 (-0.50 to -0.33) (p<0.001)	2.69 (2.63 to 2.75) (Rosi) vs 2.50 (2.44 to 2.55)(MTF) (p <0.001) 2.69 (2.63 to 2.75)(Rosi) vs 2.57 (2.51 to 2.64)(MTF) (p=0.008)
RECORD	2009	*MI* 64 (Rosi) vs 56 (Comp) § HR 1.14 (0.80-1.63) (p= 0.47)	7 (Rosi) vs 10 (Comp) § b	46 (Rosi) vs 63 (Comp) § HR 0.72 (0.49-1.06) (p=0.10)	0 (Rosi) vs 5 (Comp) § b	*Overall death* 136 (Rosi) vs 157 (Comp) § HR 0.86 (0.68-1.08) (p= 0.19) *Cardiovascular death* 60 (Rosi) vs 71 (Comp) § HR 0.84 (0.59-1.18) (p = 0.32)	*Background MTF* -0.28 (Rosi) vs +0.01 (SU) (p<0.0001) *Background SU* -0.44 (Rosi) vs -0.18 (MTF) (p<0.0001)	*Background MTF* -0.33 (Rosi) vs -0.5 (SU) (p=0.0001) *Background SU* -0.22 (Rosi) vs -0.53 (MTF) (p<0.0001)
PROactive	2005	*Non-fatal MI (excluding silent MI)* 119 (Pio) vs 144 (PL) § HR 0.83 (0.65-1.06) b	a	86 (Pio) vs 107 (PL) § HR 0.81 (0.61-1.07) b	a	177 (Pio) vs 186 (PL) HR 0.96 (0.78-1.18) b	- 0.8 (Pio) vs - 0.3 (PL) (p<0.0001)	+7.2% over baseline (Pio) vs +4.9% over baseline (PL) (p=0.003)

Comp, comparator; Glib, glibenclamide; glic, gliclazide; glim, glimepiride; glyb, glyburide; MTF, metformin; Pio, piogliotazone; PL, placebo; a, data unavailable; b, not compared statistically; c , not applicable; §figures expressed in terms of number of affected patients; ¶Patients with unstable/ severe angina, HF, uncontrolled HT were excluded from this study.

1.8.3 Meta-analyses and retrospective case control studies

A number of meta-analyses and retrospective studies have sought to explore the relationship between thiazolidinediones and cardiovascular disease (table 1.4). Generally speaking, these studies have confirmed the association of thiazolidinediones with HF.

A meta-analysis of data from 20191 patients recruited into 19 randomised controlled double blind studies analyzed congestive heart failure and cardiovascular mortality outcomes for rosiglitazone-treated (5 trials) and pioglitazone-treated (2 trials) patients [263]. Comparing with controls, thiazolidinedione-treated patients were at an increased risk of HF [2.3% vs 1.4%; RR 1.72 (95% CI 1.21, 2.42); $p < 0.002$]. There were no significant differences in cardiovascular mortality between treatment groups. Lago et al. did not report data for oedema and weight [263].

In a meta-analysis of 19 studies involving 16390 patients randomised to treatment with pioglitazone or placebo/active comparator, Lincoff et al. reported significantly higher rates of serious HF for pioglitazone-treated patients [2.34% vs 1.77%; HR 1.41 (95% CI 1.14, 1.76); $p = 0.002$] [264]. However, pioglitazone therapy conferred a significant reduction in the composite endpoint of death, myocardial infarction and stroke compared to a placebo/active comparator [4.4 % (pioglitazone) vs 5.7% (placebo/active comparator); HR 0.82 (95% CI 0.72, 0.94); $p = 0.005$], despite the absence of a similar relationship for each individual outcome [264]. An earlier Cochrane review of the safety profile of pioglitazone did not yield any meta-analysis of HF related data [265]. Analyzing randomized controlled trials lasting at least 24

weeks, the authors could only retrieve suitable data from the PROactive study, the results of which have been discussed earlier. However, the authors pointed out that data from the recruited studies showed that pioglitazone therapy was associated with a weight increase of up to 3.9 kg and a BMI rise of up to 1.5 kg/m^2. Moreover, pioglitazone was reported to increase the risk of significant oedema almost threefold [RR 2.86 (95% CI 2.14, 3.18); $p < 0.00001$] [265].

A meta-analysis by Clar et al. compared glycaemic control, hypoglycaemia, weight change, lipids and adverse events for studies recruiting patients randomized to treatment with insulin with/out adjunct pioglitazone [266]. Although adjunct pioglitazone therapy was again associated with a greater increase in body weight (1.4 to 4.4 kg for adjunct pioglitazone vs -0.04 to +4.9 kg for insulin-only groups), there was insufficient data for a formal meta-analysis of this relationship. Similarly, the investigators reported that mild to moderate oedema seemed to be more commonly reported for pioglitazone-treated patients, although p values were rarely reported [266]. Formal reports of HF were sparse, largely reflecting the fact that most studies were not sufficiently powered to investigate cardiovascular adverse outcomes. The authors were however able to conclude that adjunct pioglitazone therapy afforded beneficial effects on glycaemic control [a mean HbA1c reduction of 0.58% (95% CI -0.70, -0.46); $p < 0.001$] albeit at the expense of a greater risk of hypoglycaemia [RR 1.40 (95% CI 1.14, 1.73); $p < 0.002$] [266].

Both Singh et al. [149] and Richter et al. [267] analyzed data for rosiglitazone-treated patients. Focussing on randomized controlled studies of at least 24 weeks duration, the latter reported a rosiglitazone-associated increased risk of oedema [OR

2.27 (95% CI 1.83, 2.81); $p < 0.001$] [267]. Given that this meta-analysis showed moderate heterogeneity, the authors carried out a sensitivity analysis excluding the largest study at the time (ADOPT [154]); this reported that rosiglitazone therapy is associated with an OR for oedema of 6.04 (95% CI 3.31, 11.2) ($p < 0.00001$) [267]. Richter et al. also reported that rosiglitazone therapy was associated with a body weight increase of up to 5 kg in 11 studies and a BMI increase of up to 1.5 kg/m^2 in four studies [267]. The authors concluded that only the ADOPT study [154] yielded sufficient data for HF, diabetes-related outcomes, and overall mortality [267]. This contrasts with the approach taken by Singh et al. [149]. Analyzing data from randomized controlled studies which included at least 12 months of rosiglitazone therapy follow-up, the authors concluded that rosiglitazone therapy is associated with an increased risk of HF [1.59% of rosiglitazone-treated patients vs 0.79% of control-treated patients; RR 2.09 (95% CI 1.52, 2.88); $p < 0.001$] and myocardial infarction [1.46% of rosiglitazone-treated patients vs 1.05% of control-treated patients; RR 1.42 (95% CI 1.06, 1.91); $p = 0.02$]. The meta-analysis additionally reported no difference in cardiovascular mortality between rosiglitazone and control-treated patients [149]; the latter result generally agrees with that reported by the two largest prospective rosiglitazone studies to date (ADOPT [154] and RECORD[153]).

Despite their undisputed relevance in secondary medical research, meta-analyses need to be interpreted with caution, particularly as the resulting data are bound to guide patient management. An interesting study by Friedrich et al. showed that different methodological approaches to the rosiglitazone cardiovascular safety related meta-analyses can yield increased or decreased risks that are statistically significant or not significant at the $p = 0.05$ level [268]. An editorial by Farkouh and

Fouster maintained that p values hovering around 0.05 should be regarded with extreme caution. Indeed, some experts believe that values of 0.01 or lower should be adopted [269]. It is widely accepted that the reliability of a meta-analysis is linked to the overall number of events accrued. This is particularly of relevance when the meta-analysis includes data from predominantly small studies, as is the case with a considerable number of thiazolidinedione-related studies. In conclusion, while meta-analysis generates valuable information related to the direction of treatment effects, the mainstay of evidence based medicine relies on the outcomes of large, sufficiently powered, well-designed, randomized controlled studies [269].

In a retrospective nested case control analysis of patients on a health care database in Ontario (Canada), Lipscombe et al. studied the association between thiazolidinedione therapy and congestive HF, myocardial infarction and mortality among T2DM patients aged 66 years or older, by comparing outcomes with similarly aged individuals on other oral hypoglycaemic agents [270]. Analyzing emergency department visit and hospital admission data, the authors concluded that treatment with thiazolidinediones was associated with increased risk of HF, and that the risk was higher for those on monotherapy [adjusted RR 1.60 (95% CI 1.21, 2.10); $p < 0.001$] than those on combination therapy (ie thiazolodinediones combined with other oral hypoglycaemic agents) [adjusted RR 1.31 (95% CI 1.17, 1.47); $p < 0.001$]. Although the authors suggested that the increased risk was limited to patients treated with rosiglitazone, both as monotherapy [adjusted RR 1.98 (95% CI 1.44, 2.72); $p < 0.001$] or as part of combination therapy [adjusted RR 1.43 (95% CI 1.25, 1.63); $p < 0.001$], they indicated that their study may have been not sufficiently powered to identify a similar association for pioglitazone-treated individuals. Past

thiazolidinedione use was also associated with an increased risk of HF [adjusted RR for rosiglitazone 1.87 (95% CI 1.53, 2.28); $p < 0.001$]; the authors ascribed this to residual effects of the drug or to discontinuation in individuals with a past history of HF. While thiazolidinedione monotherapy was also associated with an increased risk of acute myocardial infarction [adjusted RR 1.40 (95% CI 1.05, 1.86), $p = 0.02$] and death [adjusted RR 1.29 (95% CI 1.02, 1.62); $p = 0.03$], combination therapy was only associated with an increased risk of the latter [adjusted RR 1.24 (95% CI 1.11, 1.39); $p < 0.001$]. As for the risk of HF, these associations were restricted to rosiglitazone-treated individuals.

In a retrospective cohort study of 91251 diabetes patients, Tzoulaki et al. analyzed the association of oral anthyperglycaemic pharmacotherapy with incident myocardial infarction (n = 3588), incident congestive HF (n = 6900) and death (n = 18548) [271]. Individually, rosiglitazone monotherapy, rosiglitazone combination therapy and pioglitazone therapy (monotherapy + combination therapy) did not show any significant association with incident myocardial infarction when compared with metformin.monotherapy, irrespective of the Cox regression model used. Neither thiazolidinedione was associated with a significantly increased risk of incident HF in the fully adjusted model, irrespective of its use as monotherapy or combination therapy. Pioglitazone therapy (alone + combined) was associated with a reduced risk of all cause mortality compared with metformin [HR 0.69 (95% CI 0.49, 0.98); $p = 0.024$] in the fully adjusted model. The authors compared the cardiovascular risks of the two thiazolidinedione drugs, reporting no significant risk differences for myocardial infarction (albeit a trend towards a higher risk with rosiglitazone). Although Tzoulaki et al. suggest that rosiglitazone is associated with a higher risk of

all-cause mortality, the reported 95% confidence intervals (CI) span unity in the fully adjusted model [HR 1.34 (95% CI 0.90, 1.97)] [271], rendering the conclusion dubious. While the overall results are reassuring, the thiazolidinedione-related data borne out of this retrospective study must be interpreted with caution – indeed the authors acknowledge the possibility of false negative results owing to a marked reduction in sample size in the fully adjusted model, such that each thiazolidinedione-associated statistical outcome was based on the analysis of less than 90 incident cases.

A retrospective analysis of electronic health data from a cohort of 20450 T2DM patients reported no differences in risk of CHF (defined via ICD-9 code and/or a post-baseline left ventricular ejection fraction <40%) between initial rosiglitazone monotherapy and initial metformin monotherapy, while suggesting an increased risk with initial pioglitazone monotherapy [HR 1.38 (95% CI 1.0, 1.90); p = 0.05] [272]. The former finding is consistent with the results of ADOPT [154]. There was no difference in CHF risk between initial rosiglitazone therapy and initial sulphonylurea therapy. Similarly, Pantalone et al. did not report differences between initial pioglitazone monotherapy and initial sulphonylurea therapy [272]. The equivalence of CHF risk for initial rosiglitazone and sulphonylurea monotherapy contrast with those reported in ADOPT [154]. Moreover, Pantalone et al. reported that initial metformin monotherapy was associated with a 24% reduction in the risk of CHF compared with initial sulphonylurea monotherapy [HR 0.76 (95% CI 0.64, 0.91); p = 0.003] [272].

Another retrospective cohort study sought to investigate the incidence of CHF among male T2DM patients seen in the South Central U.S. Veterans Administration health care network between 1st October 1996 and 31st December 2004 (n = 3956) [273]. Bivariate analysis showed that the risk of CHF was increased by a history of peripheral vascular disease ($p < 0.0001$) and higher levels of BMI ($p < 0.0001$), HbA1c ($p < 0.0001$), low-density lipoprotein ($p = 0.0002$), triglycerides ($p < 0.0001$) and systolic blood pressure ($p < 0.0001$). Prescription of a higher total number of glucose lowering agents ($p < 0.0001$), prescription of metformin ($p<0.0001$), exposure to HMG-CoA reductase inhibitors ($p < 0.0001$) and (surprisingly) treatment with thiazolidinediones ($p < 0.0001$) was associated with a lower risk of incident CHF. After adjustment for multiple cardiac risk factors, prescription of thiazolidinediones remained a lower risk factor for incident CHF [HR 0.69 (95% CI 0.60, 0.79)] [273]. In addition to the limitations imposed by a retrospective design in which treatment assignment was neither random nor blinded, the investigators acknowledged that the results of this study may have been influenced by prescribing practice, such that thiazolidinedione exposure was limited among patients perceived to be at an increased risk of HF. Additionally, Toprani et al. had no access to data showing duration of diabetes, length of treatment with thiazolidinediones and the reason for drug withdrawal. The latter may have biased study outcomes if patients developing signs of early fluid retention were withdrawn from thiazolidinedione therapy before they developed CHF as defined in the study. Moreover, a diagnosis of HF based on ICD-9 criteria may have been based on the presence of oedema or dyspnoea rather than a formal assessment of cardiac function. Finally, the study was carried out in male veterans (mean age 61.5 years), limiting extrapolation of results to female patients, and individuals in other age groups.

Habib et al. published a large retrospective cohort study of 39736 T2DM patients aged 66 years or older who were prescribed thiazolidinedione therapy between 1st April 2002 and 31st March 2008 yielded comparative data on incident HF among rosiglitazone and pioglitazone-treated patients [274]. Adjusting for demographic and clinical factors and drug doses, pioglitazone-treated patients were reported to be at a lower risk of developing the composite outcome of death or hospital admission for either acute myocardial infarction or HF than their rosiglitazone-treated counterparts [adjusted HR 0.83 (95% CI 0.76, 0.90)]. Pioglitazone therapy was also associated with lower rates of incident congestive HF and all-cause death [adjusted HR 0.77 (95% CI 0.69, 0.87) for HF; adjusted HR 0.86 (95% CI 0.75, 0.98) for mortality], despite no significant differences in the risk for myocardial infarction [adjusted HR 0.95 (95% CI 0.81, 1.11)]. Compared with high dose rosiglitazone, low dose rosiglitazone was not associated with a significant lower risk of the composite outcome [adjusted HR 0.94 (95% CI 0.83, 1.07)], whereas both low dose [adjusted HR 0.83 (95% CI 0.70, 0.97)] and high dose pioglitazone [adjusted HR 0.76 (95% CI 0.66, 0.88)] were [274].

Using time-updated propensity score adjusted analysis (modelling the probability of being treated with a thiazolidinedione), Habib et al. examined data from 19171 T2DM patients treated with oral glucose lowering agents and followed longitudinally within a US health system between 1st January 2000 and 1st December 2006 [275]. The authors compared rates of hospitalization for congestive HF between thiazolidinedione-treated patients and those not exposed to these drugs, concluding that the former were at a greater risk of CHF hospitalization [adjusted HR with

propensity adjustment (PA) 1.24 (95% CI 1.07, 1.44)] but a significantly lower risk of all-cause mortality [adjusted HR with PA 0.69 (95% CI 0.52, 0.90)]. Thiazolidinedione use was not associated with an increased risk of the composite endpoint of fatal and nonfatal acute myocardial infarction [adjusted HR with PA 0.92 (95% CI 0.73, 1.17)]. Similarly, thiazolidinedione exposure did not increase the risk of any of the other secondary outcomes, namely cerebrovascular accidents /transient ischaemic attacks [adjusted HR with PA 0.97 (95% CI 0.79, 1.20)] or combined coronary heart disease events [adjusted HR with PA 0.92 (95% CI 0.77, 1.10)]. Rosiglitazone exposure was associated with an increased risk of CHF hospitalization [adjusted HR with PA 1.65 (95% CI 1.25, 2.19)] but no significant effects on acute myocardial infarction [adjusted HR with PA 1.06 (95% CI 0.66, 1.70)], cerebrovascular events/transient ischaemic attacks [adjusted HR with PA 1.20 (95% CI 0.79, 1.82)], combined coronary heart disease events [(adjusted HR with PA 1.22 (95% CI 0.91, 1.63)] or all-cause mortality [adjusted HR with PA 0.91 (95% CI 0.57, 1.48)]. Pioglitazone treatment carried an increased risk of CHF hospitalization when analysed without propensity adjustment [adjusted HR 1.25 (95% CI 1.05, 1.50)]; this risk disappeared once the probability of being treated with pioglitazone was factored into the model [adjusted HR with PA 1.14 (95% CI 0.96, 1.37]. Pioglitazone was associated with a reduction in all-cause mortality [adjusted HR with PA 0.60 (95% CI 0.42, 0.96)], but no significant effects on acute myocardial infarction [adjusted HR with PA 0.91 (95% CI 0.69, 1.21)], cerebrovascular events/transient ischaemic attacks [adjusted HR with PA 0.93 (95% CI 0.72, 1.20)] or combined coronary heart disease events [adjusted HR with PA 0.86 (95% CI 0.69, 1.06)]. Comparing outcomes between pioglitazone- and rosiglitazone-treated patients, Habib et al. concluded that exposure to pioglitazone is

generally associated with a lower risk than rosiglitazone for all the above outcomes, although the difference only reached statistical significance for CHF hospitalizations ($p = 0.013$) and combined coronary heart disease events ($p = 0.048$) [275].

Table 1.4 - Meta-analyses and major retrospective analyses of thiazolidinedione related outcomes and side-effect profile.

Author	*Year*	*Design*	*Study inclusion criteria*	*Number of patients (trials)*	*TZD*	*Evidence of heterogeneity*	*Primary endpoint*	*Effect on primary endpoint*	*Effect on heart failure*	*Oedema*	*Effect on weight*	*Effect on IHD*	*Effect on stroke*	*Effetc on mortality*
Lincoff et al.	2007	meta-analysis	double-blind, randomized, controlled with PL/aComp	16390 (19)	Pioglitazone	no evidence	composite of death, MI or stroke	decrease 4.4% (Pio) vs 5.7% (PL/aComp)[a] HR 0.82 (0.72, 0.94)[b] p=0.005	*Serious HF* increase 2.34% (Pio) vs 1.77% (PL/aComp)[a] HR 1.41 (1.14., 1.76) [b] p=0.002	c	c	*MI* none 1.53% (Pio) vs 2.03% (PL/aComp)[a] HR 0.81 (0.64, 1.02)[b] p=0.08	none 1.22 % (Pio) vs 1.67 % (PL/aComp)[a] HR 0.80 (0.62, 1.04)[b] p=0.09	none 2.44% (Pio) vs 2.86% (PL/aComp) HR 0.92 (0.76, 1.11)[b] p= 0.38
Singh et al.	2007	meta-analysis	randomized controlled, at least 12 months of follow-up	14291 (4)	Rosiglitazone	no evidence	MI, HF and cardiovascular mortality	d	increase 1.59% (Rosi) vs 0.79 % (CL) [e] RR 2.09 (1.52, 2.88) [a] p<0.001	c	c	*MI* increase 1.46% (Rosi) vs 1.05% (CL)[e] RR 1.42 (1.06, 1.91)[b] p=0.02	c	*cardiovascular mortality* none 0.92% (Rosi) vs 0.91% (CL) RR 0.90 (0.63, 1.26)[b] p = 0.53
Lago et al.	2007	meta-analysis	randomized, controlled, double-blind	20191 (7)	Rosiglitazone (5 trials) and Pioglitazone (2 trials)	no evidence	development of congestive HF and risk of cardiovascular death	d	2.3% (TZD) vs 1.4% (comp)[e] RR 1.72 (1.21, 2.42) [b] p = 0.002	c	c	c	c	*cardiovascular mortality* none 0.7% (Rosi) vs 0.7% (Comp)[e] RR 0.93 (0.67, 1.29)[b] p = 0.68

Table 1.4 continued - Meta-analyses and major retrospective analyses of thiazolidinedione related outcomes and side-effect profile

Author	*Year*	*Design*	*Study inclusion criteria*	*Number of patients (trials)*	*TZD*	*Evidence of heterogeneity*	*Primary endpoint*	*Effect on primary endpoint*	*Effect on heart failure*	*Oedema*	*Effect on weight*	*Effect on IHD*	*Effect on stroke*	*Effect on mortality*
Lipscombe et al.	2007	retrospective case control	diabetes patients 66 years or older, 1/more OHA, 2002-2005 and followed up until 31/03/2006	159026 ([f])	Rosiglitazone and pioglitazone	f	emergency department visit or hospitalization for congestive HF	d	*hospitalization /ED visit* increased risk with TZD[h] *TZD monotherapy* aRaR 1.60 (1.21, 2.10)[b] p<0.001[e g h] *TZD combination therapy* aRaR 1.31 (1.17, 1.47) p <0.001 [e g h]	c	c	*Hospitalization /EDvisit* increased risk with TZD monotherapy[h] aRaR 1.40 (1.05, 1.86)[b] p = 0.02[eg]	c	increased risk with TZD[h] *monotherapy* aRaR 1.29 (1.02, 1.62) p=0.03[eg] *combination therapy* aRAR 1.24 (1.11-1.39) p< 0.001

Table 1.4 continued - Meta-aanlysis and major retrospective analyses of thiazolidinedione related outcomes and side-effect profile

Author	*Year*	*Design*	*Study inclusion criteria*	*Number of patients (trials)*	*TZD*	*Evidence of heterogeneity*	*Primary endpoint*	*Effect on primary endpoint*	*Effect on heart failure*	*Oedema*	*Effect on weight*	*Effect on IHD*	*Effect on stroke*	*Effect on mortality*
Clar et al.	2009	meta-analysis	trials comparing pioglitazone + insulin with same insulin regimen alone	3092 (8)	pioglitazone	no evidence (unless indicated)	glycaemic control, hypoglycaemia, wt change, lipids, adverse events	*glycaemic control* lower HbA1c for Pio + insulin -0.58% (-0.70, -0.46)[b] p<0.0001[a] *hypoglycaemia*[j] increased for Pio + Insulin RR 1.40 (1.14, 1.73)[b] p= 0.002[a j] *wt change* [c] *TC, LDL* No difference	i	commoner for Pio + insulin[i]	average weight gain of 3kg in with Pio[i]	i	c	c

Table 1.4 continued - Meta-analyses and major retrospective analyses of thiazolidinedione related outcomes and side-effect profile

Author	*Year*	*Design*	*Study inclusion criteria*	*Number of patients (trials)*	*TZD*	*Evidence of heterogeneity*	*Primary endpoint*	*Effect on primary endpoint*	*Effect on heart failure*	*Oedema*	*Effect on weight*	*Effect on IHD*	*Effect on stroke*	*Effect on mortality*
Richter et al.	2006	meta-analysis	randomized controlled, lasting at least 24 weeks	6200 (22)	pioglitazone	no evidence unless indicated	all-cause and diabetes-related morbidity and mortality, adverse events	'somewhat lower rates of hypoglycaemia'; hypoglycaemia commoner with Pio + insulin[i]	*HF requiring hospital admission* data only for PROactive (Dormandy et al.)[i]	RR 2.86 (2.14, 3.18) p<0.00001	Pio increases wt by up to 3.9 kg and BMI up to 1.5kg/m^2	data only for PROactive (Dormandy et al.)[i]	data only for PROactive (Dormandy et al.)[i]	data only for PROactive (Dormandy et al.)[i]
Richter et al.	2009	meta-analysis	randomized controlled, lasting at least 24 weeks	3888 (18)	rosiglitazone	no evidence unless otherwise indicated	all-cause and diabetes-related morbidity and mortality, adverse events	'somewhat lower rates of hypoglycaemia' with Rosi, 'especially when compared to SU'; 'severe hypo were rarely reported'[i]	data only for ADOPT (Kahn et al.)[i]	increased risk with Rosi OR 2.27 (1.83, 2.81) p<0.00001[k] *excluding Kahn* et al.*:* OR 6.04 (3.31, 11.2) p<0.00001[l]	Rosi increased weight by up to 5kg in 11 studies and BMI by up to 1.5kg/m^2 in 4 studies	data only for ADOPT (Kahn et al.)[i]	data only for ADOPT (Kahn et al.)[i]	data only for ADOPT (Kahn et al.)[i]

aComp, active comparator; aRaR, adjusted rate ratio; Comp, comparator; CL, control; ED, emergency department; HR, hazard ratio; pio, pioglitazone; PL, placebo; rosi, rosiglitazone; RR, relative risk; TC, total cholesterol;/ LDL, low-density lipoprotein cholesterol; OR, odds ratio; [a] number of affected patients; [b] 95% confidence intervals; [c]data unavailable; [d] data included in adjacent columns; [e] event rate; [f] not applicable; [g] comparing TZD therapy with other oral hypoglycaemic agent combination therapies; [h] risk appeared limited to rosiglitazone use; [i] meta-analysis not possible; [j] sensitivity analysis showing moderate heterogeneity; [k] sensitivity analysis showing heterogeneity (I^2 = 53.4%); [l] sensitivity analysis showing no significant heterogeneity.

1.9 Association of comparator 'first and second line' oral glucose lowering agents (metformin, sulphonylureas) with incident heart failure

Evidence supporting or refuting a possible association between other glucose lowering agents and incident HF is surpisingly sparse. Although the US Food and Drug Administration relatively recently removed its contraindication to prescribing metformin in patients with HF, it strongly cautions its use in this setting. This clinical concern is likely to account for an absence of randomised control trials exploring outcomes in metformin-treated T2DM individuals with, or prone to HF. The only prospective data exploring incident HF events in metformin-treated patients were provided by the RECORD study [153]. Three retrospective studies recruiting patients from a US register of T2DM patients shed valuable information in this regard. Nichols et al. reported that incident congestive HF rates were lowest in regimens that included metformin and highest in those that included insulin. Compared with patients on metformin monotherapy (typical ‘early stage’ diabetes), adjusted incident congestive HF rates (per 1000 patient years) were 32% higher among patients treated with sulphonylurea monotherapy, 28% higher among patients on metformin-sulphonylurea combination therapy, and 2.6 times higher in patients on insulin monotherapy [276]. These findings are consistent with those from an earlier retrospective study [277]. In contrast, use of metformin or sulphonylureas did not influence incident congestive HF rates over a follow-up period of 72 months, unlike insulin [HR 1.25 (95% CI 1.06, 1.48); $p < 0.001$] [239]. Analyzing data from 6900 incident cases of congestive HF occurring in 91521 patients with T2DM who were followed up for a mean period of 7.1 years, Tzoulaki et al. reported that, compared with metformin monotherapy, second generaton sulphonylurea

monotherapy was associated with an 18% to 30% excess risk of new onset congestive HF in adjusted Cox regression models. Rosiglitazone combination therapy (with meformin and/or sulphonylurea) was associated with an increased risk of incident HF compared with individuals prescribed metformin monotherapy in two Cox regression models. Neither sulphonylureas (first or second generation) nor thiazolidinediones rosiglitazone or pioglitazone) emerged as significant risk factors for new onset congestive HF in a fully adjusted model [271].

The effect of duration of therapy on incident HF rates was investigated by Maru and colleagues. Analyzing data from 25, 690 newly diagnosed T2DM patients registered in the UK General Practice Research Database, glucose lowering agent use (metformin or sulphonylurea or insulin) within the first year of diagnosis carried a 4.75 fold (hazard ratio) increased risk of incident HF compared with their drug free counterparts. This risk did not persist beyond the first year (mean follow-up 2.5 years) and seemed unrelated to type-specific drug exposures [278].

A retrospective study recruiting 5631 T2DM patients newly treated with a single oral glucose lowering agent and followed up for almost five years further supported evidence for an association between high sulphonylureas and incident HF [adjusted HR 1.24 (95% CI 1.01, 1.54)]. Additionally, McAlister and colleagues reported that high dose sulphonylurea therapy was more likely to result in incident HF [HR 1.38 (95% CI 1.20, 1.60)] than low dose sulphonylureas. No such association existed for metformin users [279].

1.10 Use of comparator 'first and second' line oral glucose lowering agents (metformin, sulphonylureas) in patients with established heart failure

Compared with data for incident HF, a larger number of studies (mostly observational) looked at additional HF events and outcomes in patients with T2DM and established HF. Using propensity score matched samples, Aguilar et al. reported that metformin therapy was associated with lower mortality rates [HR 0.76 (95% CI 0.63, 0.92)], albeit no effect on hospitalization rates [280]. A retrospective analysis of data from 12 272 HF patients who were newly prescribed with oral glucose lowering agents for T2DM reported that both metforin monotherapy and metformin-sulphonylurea combination therapy were associated with fewer deaths than sulphonylurea monotherapy [adjusted HR 0.70 (95% CI 0.54, 0.91) and 0.61 (95% CI 0.52, 0.72) respectively] [281]. A reduction in the composite of all-cause deaths or all-cause hospitalizations was also observed [HR 0.83 (95% CI 0.70, 0.99) for metformin monotherapy vs sulphonylurea monotherapy; HR 0.86 (95% CI 0.77, 0.96) for metformin monotherapy vs metformin-sulphonylurea combination therapy]. Risks of all-cause death, all-cause hospitalization and the composite (all-cause hospitalization or all-cause death) seemingly increased at study end (mean ± SD duration of follow-up 2.5 ± 2 years) compared with results at one year [281]. These results generally agree with those reported by Andersson et al. in a cohort of Danish patients treated with metformin, sulphonylureas or insulin in the setting of established HF. Using sulphonylurea monotherapy as a reference, these authors reported that metformin monotherapy carries the lowest mortality risk in this setting [adjusted HR 0.85 (95% CI 0.75, 0.98)] followed by metformin-sulphonylurea combination therapy [adjusted HR 0.89 (95% CI 0.82, 0.96)] and insulin [adjusted

HR 1.14 (95% CI 1.06, 1.20)] [282]. A retrospective review of 16 417 T2DM with established HF showed that treatment with both metformin [adjusted HR 0.86 (95% CI 0.78, 0.97)] and thiazolidinediones [adjusted HR 0.87 (95% CI 0.80, 0.94)] was associated with a lower risk of death compared with patients not treated with an insulin sensitizer (sulphonylurea or insulin). Readmission with HF was more likely in patients treated with a thiazolidinedione [adjusted HR 1.06 (95% CI 1.00, 1.09)] and less likely in patients on metformin [adjusted HR 0.92 (95% CI 0.86, 0.99)] [283].

A systematic review and meta-analyses of randomised studies or controlled trials revealed that metformin significantly reduced all cause mortality in two studies [HR 0.86 (95% CI 0.78, 0.97)] compared with other anitdiabetic drugs and insulin; a similar trend was seen in a third study. Metformin was also associated with reduced all cause hospital admissions at one year compared to other treatments [pooled OR 0.85 (95% CI 0.76, 0.95); p = 0.004] [284]. In 1633 patients newly diagnosed with T2DM and HF, both metformin monotherapy [adjusted OR 0.65 (95% CI 0.48, 0.87)] and metformin combined with/out other agents [adjusted OR 0.72 (95% CI 0.59, 0.90)] were associated with reduced mortality rates compared with antidiabetic treatment naïve patients [285].

In conclusion, both prospective and retrospective studies support an association between thiazolidinediones and oedema/heart failure. Absolute rates may be higher in the setting of cardiovsacular disease, and are possibly influenced by the concurrent use of other glucose lowering pharmacotherapies.

Section III - Mechanisms underpinning fluid retention following thiazolidinedione therapy

While the association between thiazolidinedione therapy, cardiac failure and fluid retention has been demonstrated by several prospective and retrospective studies/meta-analyses, the pathophysiological mechanisms underlying these complications remain unclear. It is clear that the current paucity of research data in this field impairs the identification of any predisposing factors of thiazolidinedione induced fluid overload, an issue which hampers the development of clearer clinical guidelines governing their use. Moreover, concerns regarding the cardiovascular safety profile of rosiglitazone, initially reported in Nissen and Wolski's meta-analysis [147] and culminating in an FDA's ruling (since revised) that this drug increases cardiovascular events [150, 160], influenced prescribing practices worldwide. Indeed, this has been outlined in a recent study of prescribing data in Tayside, Scotland for the period October 2006-March 2008, which confirmed a 34% decrease in the number of prescriptions for rosiglitazone (alone or as combination therapy with metformin), and an accompanying increase in those for pioglitazone (alone or as combination with metformin) [286]. These developments, call for a concerted effort in this regard towards a better understanding of the relevant mechanisms.

1.11 Renal haemodynamics

As outlined earlier, the PPAR-γ1 receptor isotype has been shown to be moderately expressed in the kidneys. Guan et al. examined the distribution of the different PPAR

receptors within the human kidney and urinary tract using in situ hybridization techniques, concluding that renal PPAR-γ receptors are exclusively expressed in the medullary collecting duct, ureter and bladder [287]. PPAR-γ is also expressed to a lesser extent in the glomeruli and renal microvasculature [288]. Low but significant expression has been reported in the proximal tubules and in many other nephron segments [289]. Other studies have reported constitutive expression of PPAR-γ receptors in cultured glomerular mesangial cells, podocytes, proximal epithelial cells and epithelial cells of collecting ducts [290]. This distribution suggests diverse roles for PPAR-γ in the kidney, both therapeutically and in its modulation of thiazolidinedione-induced fluid overload.

1.11.1 The collecting duct and distal tubule

The localisation of PPAR-γ receptors in the medullary collecting duct lead to the hypothesis that PPAR-γ activation increases sodium retention through its action at this critical site in fluid metabolism that responds to the integrated effects of multiple hormones such as aldosterone, arginine vasopressin (AVP), insulin and atrial natriuretic peptide (ANP) [291]. Acting via the mineralocorticoid receptor, aldosterone enhances the absorption of sodium by the principal cells of the collecting duct. This is achieved by inducing the expression of key genes that encode for key regulators of sodium transport, namely the epithelial sodium channel-α (ENaCα,), serum and glucocorticoid regulated kinase-1 (Sgk) and the sodium-potassium-ATPase-α (Na-K-ATPase-α) [292, 293] (table 1.5). Reabsorption of sodium in the distal nephron is a two-step process. Sodium first enters renal cells from the luminal compartment via the rate-limiting apical ENaC, and is then actively transported out

of the cell by the basolateral Na-K-ATPase [294]. ENaC consists of three subunits designated ENaC- α, -β and -γ [295]. Expression of the ENaCγ subunit (encoded by the Scnn1g gene) plays a crucial role in the trafficking of the ENaCα, and ENaCβ to the cell membrane [296]. Sgk is a novel member of the serine/threonine kinase gene family, comprising three highly organ-specific isoforms (Sgk-1, -2, -3) sharing 80% amino acid identity [297, 298]. Skg-1 is thought to be a key mediator of aldosterone-induced sodium reabsorption through the ENaC at the collecting duct [299], and has been reported as a target gene of PPAR-γ in a murine study [291]. Similarly, Hong et al. have shown that PPAR-γ can bind to specific elements in the Sgk-1 promoter in human collecting duct cells [300]. Hypotonic conditions increase Sgk-1 expression and sodium transport in A6 cells, a cultured cell line derived from the Xenopus laevis distal nephron [301]. This contrasted with findings by Guan et al., who did not find any evidence for increased expression of Sgk-1 in cultured mouse inner medullary collecting duct cells. [302]

Two elegant murine studies sought to investigate the hypothesis that thiazolidinediones induce fluid retention through PPAR-γ mediated activity at the collecting duct. Deletion of PPARg (which encodes for PPAR-γ) in the murine collecting duct prevented thiazolidinedione-induced weight gain, decreased renal sodium retention and increased plasma aldosterone (a reliable index of plasma volume) in a study by Guan et al. [302]. Mice pre-treated with amiloride (an aldosterone antagonist) at a dose of 2mg/kg/day were also immune to the weight increasing effect of pioglitazone. Additionally, the authors reported that the treatment of cultured collecting ducts with thiazolidinediones increased amiloride-sensitive sodium absorption through the epithelial sodium channel (ENaC); this

effect was abolished in PPAR-γ deficient collecting duct cells. Guan et al. [302] demonstrated that Scnn1g expression is increased by thiazolidinedione therapy, identifying Scnn1g as a direct and specific target gene of PPAR-γ in the medullary collecting duct. A contemporary study by Zhang et al. comparing outcomes in PPAR-γ collecting duct knock-out and control mice reported similar thiazolidinedione-induced PPAR-γ mediated differences in body weight, sodium balance, ENaC sodium transport and plasma aldosterone levels [303].

The data from the above two studies somewhat contrast with those from another study investigating the renal effects of the highly potent and selective PPAR-γ agonist farglitazar [291]. Murine administration of this pharmacological agent led to plasma volume expansion, a small but significant decrease in plasma potassium, lower aldosterone concentrations and a small but significant increase in plasma sodium and chloride concentrations. These changes are consistent with aldosterone's role at the level of the medullary collecting duct, favouring sodium reabsorption and potassium excretion. Paradoxically however, low dose amiloride (1mg/kg/day) exacerbated farglitazar-induced plasma volume expansion and significantly increased the renal expression of ENaCα. One notes however that the investigators used a lower dose of amiloride in this study (1mg/kg/day) compared with Guan et al. [302], which may, at least in part, explain the difference in treatment outcomes.

Artunc et al. compared body weight, haematocrit, plasma aldosterone, leptin, blood pressure and renal Sgk-1 expression in Sgk knockout mice and their wild type littermates treated with pioglitazone [304]. Pioglitazone treatment significantly increased Sgk-1 mRNA and protein expression and plasma volume only in wild type

mice. The latter group also exhibited a significantly greater increase in in body weight and a significantly more pronounced reduction in haematocrit in response to treatment. Pioglitazone therapy decreased plasma aldosterone and blood pressure, and increased leptin levels in both litter genetic subtypes. The authors concluded that Sgk-1 contributes, but does not fully explain thiazolidinecdione-induced fluid retention.

Nofziger et al. reported no change in Sgk-1 transcript or protein expression after incubating mouse principal kidney cortical collecting duct cells with the PPAR-γ agonists GW7845 (a potent non-thiazolidinedione) and pioglitazone [305]. Although the authors were able to identify PPAR-γ in 3 different *in vitro* models of renal principal cells, the same agents did not increase basal or insulin-stimulated sodium flux via the ENaC, supporting the possibility that these agonists may be favouring water and sodium retention at a more proximal site within the nephron. Consistent with these observations, Vallon et al. reported that mice selectively lacking the ENaC α subunit in the collecting duct were still prone to thiazolidinedione-induced water retention, and that thiazolidinediones increased the activity of an unspecified nonselective cation channel [306]. Indeed, in a recent publication, the latter research group found that thiazolidinediones may actually repress the ENaC γ subunit transcription by suppressing histone H4K5 acetylation in murine M1 collecting duct cells [307].

1.11.2 The proximal tubule

As discussed earlier, PPAR-γ is also reportedly expressed in human proximal tubular cells. Moreover, this expression is up-regulated in the presence of high glucose and PPAR-γ agonists [308]. The proximal tubule constitutes more than 90% of renal tissue, and together with the descending limbs of Henle's loop, account for the reabsorption of approximately 80% of the water and solutes, and 60% of the sodium filtered at glomerular level. This is mediated through the activity of membrane-inserted water channel proteins called aquaporins (AQPs). AQP1 and AQP7 are the principal isoforms expressed in the proximal tubule [309, 310].

Strongly expressed in the apical and basolateral plasma membranes of proximal tubular cells, AQP1 plays a major role in proximal tubular transcellular transport [310-312]. Experiments on AQP1 knockout mice reduced proximal tubular transport by 90%, suggesting that 90% of water transport at the proximal convoluted tubule is transcellular and 10% is paracellular [313, 314]. Schnermann et al. concluded that other AQPs and non-AQP transporters play little, if any role in determining proximal tubule water reabsorption [314]. AQP7 is an aquaglyceroporin, which allows the rapid transport of glycerol and water; it is expressed on the apical membrane of the proximal straight tubules [315]. Murine experiments using AQP7 knockout and AQP1/AQP7 knockout mice concluded that the estimated relative contribution of AQP7 to water permeability on the proximal straight tubules was one-eight that of AQP1 [316].

Although rosiglitazone has been reported to induce AQP2 and AQP3 in whole kidney homogenates in rats and AQP1, AQP2 and AQP3 in the inner medulla [310], the effect of PPAR-γ on AQP expression in the proximal tubule is largely unknown. AQP7 has been identified as a PPAR-γ target gene [317]. Saad et al. reported that PPAR-γ agonists enhance the expression of AQP1 and AQP7 in humans through an Sgk-1 mediated pathway [318]. The clinical relevance of these findings remains unclear.

The type 3 sodium hydrogen exchanger (NHE3) is another key modulator of sodium reabsorption at the proximal tubule. Rosiglitazone was reported to increase NHE3 (and the α1 subunit of the sodium-potassium-ATPase, the bumetanide sensitive sodium-potassium-2 chloride cotransporter, aquaporins 2 and 3, and endothelial nitric oxide synthase) expression in a murine model [319]. The effect of thiazolidinediones on NHE3 expression was later confirmed in human proximal tubular cells, occurring through an Sgk-1 dependent pathway [318]. The basolateral type 1 sodium-bicarbonate cotansporter has also been implicated in thiazolidinedione-induced fluid retention, as evidenced by data from a study by Muto et al. [320]. Using *in vitro* electrophysiological studies on rabbit proximal straight tubule cells, the authors established that troglitazone stimulated this cotransporter in a dose dependent fashion. Endo et al. described similar findings in rat, rabbit, human but not in mouse proximal tubular cells. Additionally, these authors reported that stimulation of the sodium-bicarbonate cotransporter is mediated in a non-genomic fashion through PPAR-γ induced stimulation of the Src-EGFR-ERK signalling pathway [321].

Zanchi et al. investigated the effects of pioglitazone on renal salt water handling in response to a low salt (20 mmol/day) and a high salt (>200 mmol/day) diet [322]. This double-blind, randomized, placebo controlled, cross-over study recruiting 10 healthy normotensive male subjects demonstrated that a 6 week course of pioglitazone therapy (45 mg daily) significantly lowered urinary sodium excretion and reduces lithium clearance when patients were subjected to a low salt diet, suggesting that the drug increases proximal tubular sodium reabsorption. A high salt diet produced similar trends (albeit not statistically significant); the authors ascribed this to individual variability.

In summary, the physiological mechanisms underlying thiazolidinedione- induced salt and water retention in the kidney remain largely unravelled and the subject of considerable debate, despite their clear clinical importance.

Table 1.5 - Distribution of sodium transporters and sodium channel proteins in the nephron. Transporters marked with an asterisk (*) have been implicated to play a role in PPARγ mediated salt retention.

Nephron location	*Transporter/channel protein*	*Cellular location*
All locations	Na-K-ATPase*	Basolateral
Proximal tubule	Type 3 Na-H exchanger*	Apical
Proximal tubule	Type 2 Na-phosphate cotransporter*	Apical
Proximal tubule	Type 1 Na-bicarbonate cotransporter*	Basolateral
Descending limb of Henle's loop	Type 3 Na-H exchanger*	Apical
Thick ascending limb of Henle's loop	Type 3 Na-H exchanger*	Apical
Thick ascending limb of Henle's loop	Type 2 Na-K-2Cl transporter[a*]	Apical
Distal convoluted tubule	Na-Cl cotransporter[b]	Apical
Connecting tubule	Epithelial Na channel[c*]	Apical
Collecting duct	Epithelial Na channel α subunit[c*]	Apical

[a] bumetanide sensitive; [b] thiazide sensitive; [c]amiloride sensitive

1.11.3 Evidence for an 'escape mechanism' and the 'salt handling paradox'

The observation that PPAR-γ agonist treatment is associated with a lowering of blood pressure suggests the existence of an interaction with the cardiovascular system. This is further supported by the observation that patients with a dominant negative mutation in PPAR-γ exhibit early onset hypertension [323]. One of the major regulators of systemic blood pressure is the renin-angiotensin-aldosterone system. Thiazolidinedione treatment was reported to prevent an increase in blood

pressure caused by the infusion of angiotensin II in rats [324]. Additionally, it decreases blood pressure and improves endothelial function in a mouse model of lifelong hypertension caused by the overexpression of both human renin and human angiotensinogen transgenes [325]. Morerover, thiazolidinedione therapy was also reported to downregulate angiotensin II type I receptor gene expression via a PPAR-γ dependent mechanism in vascular smooth muscle cells [326].

Renin is the rate limiting step in angiotensin II synthesis. In their study on healthy normotensive male volunteers subjected to a low and high salt diet, Zanchi et al. [322] reported that pioglitazone therapy increased plasma renin activity in both salt-loading states, despite recording no significant blood pressure changes in response to thiazolidinedione treatment or alteration in dietary sodium load. However, the authors did note that piogliazone therapy was associated with a significant increase in daytime heart rate, which reached statistical significance only on a low salt diet. In the absence of any effect on supine (nocturnal) blood pressure, the authors postulated that the raised renin is a physiological response to thiazolidinedione induced peripheral vasodilatation. This hypothesis was consistent with earlier reports that thiazolidinediones exert several vasodilatory effects on the vascular system, namely reducing endothelin-1 secretion by endothelial cells [327], modulating its endogenous production in endothelin-dependent hypertension [328] and inhibiting vascular smooth muscle calcium currents [329, 330]. In a later study on human renin secreting Calu-6 cells (derived from a pulmonary carcinoma), Todorov et al. reported that rosiglitazone increases renin gene expression via a PPAR-γ dependent mechanism. This association is however disputed, since other studies have reported that PPAR-γ has no influence on renin mRNA levels [331, 332]. Despite the lack of

consistent data, a delicate balance may exist between the effects of thiazolidinediones on the renin-angiotensin-aldosterone system and other mediators of vascular tone. Further studies are warranted to clarify this relationship.

Nonetheless, these observations cannot adequately explain the dietary sodium related differences in renal salt handling during thiazolidinedione therapy [322]. Animal studies suggest that sodium reabsorption from the collecting duct during rosiglitazone treatment reaches a peak at day 6, and that balance returns to normal by day 9 [303]. This led to the hypothesis that an ‘escape mechanism’ plays a role in thiazolidinedione-associated sodium handling under salt-loading conditions, similar to that seen with mineralocorticoid excess [333, 334]. ANP is thought to play a critical role in this mechanism [333, 334], particularly in low renin states. To this effect, Goenka et al. [335] investigated the effects of water immersion to the level of the neck (which causes a 16% increase in plasma volume and a redistribution of 700 mls of blood centrally to the thoracic cavity) on renal and hormonal dynamics in normal and T2DM individuals. The investigators confirmed earlier reports that T2DM patients are characterized by an impaired natriuretic response, diminished ANP and a blunted cGMP response to volume expansion [335]. Rosiglitazone treatment for 7 days restored these responses in T2DM individuals, and significantly increased the ANP response in control individuals [335]. These findings may, at least in part, explain the salt handling differences reported by Zanchi et al. [322], such that individuals on a chronic high salt diet would have already suppressed the renin-angiotensin-aldosterone system and increased their ANP secretion, limiting further physiological responses to thiazolidinedione therapy [335].

Available data suggest that thiazolidinedione therapy paradoxically increases, rather than decreases natriuresis in response to volume expansion. Rosiglitazone treated Zucker rats (an animal model of T2DM) exposed to an acute sodium load (a volume expansion stimulus) showed a more rapid natriuresis compared to control animals [336]. Goenka et al. reported similar results in humans, suggesting that in thiazolidinedione treated individuals, the initial increased sodium retention leads to increased ANP levels or sensitivity, which in turn contributes to an enhanced natriuretic response to an acute sodium load. This may prove to be a protective mechanism against fluid retention [335].

Given that the common praline-to-alanine substitution at codon 12 (Pro12Ala) of exon B in the PPAR-γ gene may be a pharmacogenetic risk factor for thiazolidinedione-induced oedema [337], and that ACE inhibitors may be less effective in individuals with this polymorphism [338], studies are warranted to investigate whether the propensity for thiazolidinedione-induced fluid retention arises as a result of a lower state of activation of the renin-angiotensin-aldosterone system. Moreover, although Black and Asian ethnic group individuals with low renin volume mechanisms have been shown to have higher proximal tubular sodium reabsorption [339], it is unclear whether such individuals are more prone to thiazolidinedione-induced oedema.

1.11.4 Endothelial dysfunction and peripheral vascular resistance

Increased arterial stiffness and endothelial dysfunction associated with diabetes and the metabolic syndrome may in part explain the increased cardiovascular risk

associated with these conditions [340]. Biopsy specimens from subcutaneous fat have demonstrated that endothelial dysfunction [341] and structural alteration [342] of small resistance arteries contribute to peripheral vascular resistance in diabetic patients. In healthy individuals, vascular endothelial secretion of nitric oxide (vasodilator) and endothelin (vasoconstrictor) is kept in balance by circulating insulin levels [343, 344]. Hyperinsulinaemia disrupts this fine balance, favouring an enhancement of endothelin secretion and a reduction in nitric oxide secretion [344]. In turn, this increases vascular tone, arterial stiffness and peripheral vascular resistance. Not surprisingly, arterial stiffness has been identified as a risk factor for HF [345]. Moreover, arterial stiffness has recently been associated with early and asymptomatic impairment of systolic and diastolic myocardial function [346].

The mechanism by which thiazolidinediones reduce peripheral vascular resistance is likely to be multifactorial. PPAR-γ is expressed in various components of the vascular system, including endothelial cells, the vascular smooth muscle cells of the intimal and medial layers, and monocytes/macrophages [347]. As discussed earlier, thiazolidinediones downregulate endothelin-1 secretion, inhibit vascular smooth muscle currents, and are likely to restore the fine balance between circulating levels of endothelin and NO, by virtue of their insulin sensitizing effects. These drugs have also been shown to have a favourable on low grade inflammation, as evidenced, for example, by a reduction in circulating levels of plasminogen activator inhibitor-1, interleukin-6, tumour necrosis factor-α (TNF-α) and non-esterified fatty acids, all of which have been associated with insulin resistance[348], and its associated vascular endothelial dysfunction [349].

These effects cumulatively translate into a thiazolidinedione-associated improvement in arterial stiffness, as shown in clinical trials [350, 351]. This beneficial effect should theoretically reduce (rather than increase) the risk of development of HF and its associated fluid retention. Nonetheless, there are no studies evaluating whether T2DM individuals with a history of thiazolidinedione-induced HF are characterised by a greater degree of arterial stiffness compared to their 'thiazolidinedione tolerant' counterparts. Such a 'susceptiblity factor', if existent, could in turn be influenced by sodium balance, and possibly by relative thiazolidinedione-induced improvements in insulin sensitivity. The oedematogenic properties of insulin are well documented [352]. Indeed, insulin has been shown to favour sodium reabsorption along various nephron segments [353]. Blazer-Yost et al. described insulin-induced, PI3K-mediated, activation of the ENaC at the distal convoluted tubule/medullary collecting duct [354]. The PI3K pathway has also been shown to mediate insulin-induced sodium reabsorption at the proximal convoluted tubule [355, 356]. It has long been been suggested that the association of thiazolidinediones with oedema may occur on account of its favourable effects on insulin sensitivity, and may well explain why the prevalence of oedema is higher in patients treated with a combination of insulin and thiazolidinediones [357]. Other authors have ascribed this phenomenon to a synergistic effect of thiazolidinediones and insulin on renal sodium handling [358], particularly given the observation that the oedematogenic effects of insulin require IRS2 rather than IRS1 [356]. Insulin resistance is often associated with defects in the IRS1-dependent signalling, while IRS2-dependent signalling seems to be sometimes preserved in adipocytes and skeletal muscle [66, 359-361].

1.11.5 Effects on vascular permeability

Increased capillary permeability has also been postulated to contribute to thiazolidinedione-induced oedema. This hypothesis was first investigated by Idris et al. [362], who examined the effects of rosiglitazone on endothelial barrier function using an *in vitro* system of pulmonary artery endothelial cell monolayers, and Evans blue-labelled albumin to measure transendothelial albumin flux. Exposure of the cells to high concentrations of rosiglitazone (10 μM to 100 μM) for 4 hours resulted in a dose-dependent increase in transendothelial albumin flux. This effect was fully reversible on washing rosiglitazone off the monolayer, and subsided if exposure was prolonged to 24-48 hours.

The mechanism(s) underlying thiazolidinedione-induced capillary permeability remain obscure. Several factors, notably vascular endothelial growth factor (VEGF), nitric oxide and protein kinase C have been implicated.

(i) Vascular Endothelial Growth Factor (VEGF)

VEGF is estimated to be 50 times more potent than histamine in enhancing vascular permeability [363]. Lower extremity oedema was induced following gene transfer of naked plasmid DNA encoding the 165 amino-acid isoform of VEGF in patients with peripheral artery disease [364]. Emoto et al. reported that plasma levels of VEGF were significantly higher in troglitazone treated T2DM patients compared to those treated with dietary measures alone, sulphonylurea or insulin [365]. Additionally, a longitudinal study of 5 glibenclamide treated T2DM patients showed that adjunct

troglitazone therapy was associated with a reversible increase in plasma VEGF levels [365]. The same investigators reported that therapeutic concentrations of troglitazone and rosiglitazone are associated with an increase in VEGF mRNA expression in 3T3-L1 adipocytes. In a study on Zucker rats, Sotirpoulos et al. established that rosiglitazone treatment increased VEGF mRNA expression in epididymal fat, and that this correlated with increased vascular permeability [366]. Similar findings were reported in retinal tissue, although the increase in VEGF mRNA did not reach statistical significance [366]. Troglitazone, pioglitazone and two other experimental PPAR-γ agonists (LY171883 and 15d-PGI_2) increased VEGF secretion from cultured human umbilical artery vascular smooth muscle cells [367]. While these studies support a role for VEGF in thiazolidinedione-induced oedema, they are not consistent with the results of other investigations, which suggest that PPAR-γ negatively regulates VEGF signalling.

Both rosiglitazone and 15-deoxy-delta 12, 14-prostaglandin decreased VEGF protein expression in transformed and primary endometrial cells in a study by Peeters et al. [368]. Using PPRE3 luciferase reporter transfected Ishikawa adenocarcinoma cells, rosiglitazone was shown to repress VEGF promoter activity in a dose-dependent fashion (IC_{50} around 50 nM). Cotransfecting full-length and truncated VEGF promoter-luciferase constructs and PPAR-γ expression vectors into Ishikawa cells, Peeters et al. also revealed that the PPAR-γ regulated domain is a direct repeat-1 motif - 443 bp upstream of the transcriptional start site [368]. Sander et al. [369] reported that rosiglitazone inhibits VEGF-induced proliferation and migration of human pulmonary valve endothelial cells, by antagonizing VEGF-mediated nuclear factor of activated T cells, Cytoplasmic 1 (NFATc1) (essential for heart valve

formation). This inhibitory mechanism was confirmed in a parallel study on human umbilical vein endothelial cells [369]. In another study on identical cells, rosiglitazone was reported to markedly reduce VEGF-induced tube formation and endothelial cell migration, which are critical steps in angiogenesis [370]. Tooke et al. reported no significant difference in change in VEGF levels among insulin treated T2DM patients who were randomized to treatment with pioglitazone (n = 14) or placebo (n = 15) [371].

The conflicting data summarized above might result from a PPAR-γ induced, possibly cell specific, dual effect on VEGF signalling. The relative contribution of these factors, if any, in thiazolidinedione-induced oedema remains obscure.

(ii) Nitric oxide (NO)

Synthesised by endothelial cells from the amino acid L-arginine through the activity of endothelial nitric oxide synthase (eNOS), the ubiquitous naturally occurring molecule nitric oxide (NO) is an important regulator of vascular function, including vascular permeabilty. eNOS is regulated at the level of expression [372-374], post-translationally through its interaction with multiple proteins [375-378], and by eNOS phosphorylation [379-382]. A possible relationship between thiazolidinediones and NO was first reported by Vinik et al.. In a 16 week, randomized, double-blind, placebo-controlled, crossover to open-label single blind trial, NO production was significantly increased in rosiglitazone treated T2DM patients [383]. Treating human umbilical vein endothelial cells with the PPAR-γ ligands 15d-PGI_2, ciglitazone and rosiglitazone increased nitric oxide synthase (NOS) activity and NO release through

a PPAR-γ dependent mechanism in a study by Polikandriotis et al. [384]. Furthermore, the investigators reported that rosiglitazone and 15d-PGI_2 treatment lead to eNOS ser1177 phosphorylation, an effect that is attenuated by the PPAR-γ antagonist GW9962 [384]. In an *in vivo* study on the fructose-fed rat model, St-Pierre et al. investigated vascular permeability by assessing the extravasation of Evans blue dye in distinct muscle groups [385]. Rosiglitazone increased extravasation by 30-50% in the rectus femoris, soleus, gastrocnemius lateralis, vastus lateralis and tibialis cranialis skeletal muscles. In homogenates of skeletal muscles (vastus lateralis) from fructose-fed rats, rosiglitazone treatment resulted in a significant increase in NOS activity and eNOS immunoreactive mass, compared to control animals. Interestingly, the authors reported no significant change in the level of neuronal NOS (the most common muscle NOS isoform) [385].

(iii) Protein kinase C (PKC)

Protein kinase C (PKC) constitutes an important determinant of vascular permeability through its phosphorylation of cytoskeletal proteins that make up the tight intercellular junction [386-389]. In a study on Zucker rats, Sotiropoulos et al. reported that the rosiglitazone-associated increases in vascular permeability and weight were associated with selective activation of PKC and its potent activator diacylglycerol (DAG) in fat and retinal tissues [366]. The same investigators established that these rosiglitazone-induced effects in adipose tissue were abolished by the specific PKCβ inhibitor ruboxistaurin and in PKCβ knockout mice [366].

(iv) Other potential permeability factors

Analyzing 384 single nucleotide polymorphisms (SNPs) from 222 cardiovascular and metabolic genes in 87 thiazolidinedione treated T2DM patients, Ruano et al. sought to discover associations between thiazolidinedione therapy and oedema [390]. The investigators reported significant associations with the genes for neuropeptide Y, glycogen synthase-1 muscle (Gsk-1 muscle), chemokine C-C motif ligand 2, oxidized LDL receptor 1 and Growth Hormone Releasing Hormone [390]. Despite being a long-lasting vasoconstrictor, neuropeptide Y increases endothelial permeability [391], and has been implicated in neurogenic pulmonary oedema [392], laryngeal oedema [393] and inflammatory paw oedema in rats [394]. Chemokine C-C motif ligand 2 increases the permeability of the blood-brain barrier, and contributes to vasogenic brain oedema [395]. Encoded by the ORL1 gene, the oxidized LDL receptor 1 is expressed on vascular endothelial cells [396] and is involved in capillary formation [397]. In summary, Ruano et al. conclude that the physiogenomic associations suggest a link between vascular permeability and thiazolidinedione-induced oedema [390].

1.12 Thiazolidinediones and cardiac pump function

Partients with diabetes have a high prevalence of subclinical systolic and diastolic cardiac dysfunction and impaired cardiac reserve, likely due to a number of abnormalites such as impaired coronary flow reserve, even in the absence of obstructive epicardial disease [398, 399], autonomic dysfunction [400-402], myocardial fibrosis [403] and maladaptive myocardial energy metabolism [404].

Although cardiac expression of PPAR-γ is relatively lower than PPAR-α, the former is thought to be an important modulator of cardiac structure and function, particularly in the left ventricle. In a murine study by Duan et al., cardiac-specific deletion of the PPAR-γ receptor resulted in mild cardiac hypertrophy [405]. These findings were consistent with earlier reports that the pressure-overload induced increases in heart weight-to-body weight ratio and wall thickness were more prominent in heterozygous PPAR-γ deficient mice compared to their wild type counterparts [406].

The effects of exogenous PPAR-γ treatment on cardiac function is controversial. Studies have shown that PPAR-γ agonist therapy inhibits mechanical strain- [407], angiotensin-II- [406, 407] and phenylephrine-induced [407] cardiac hypertrophy of neonatal cardiac rat myocytes *in vitro*. Asakawa et al. reported similar results *in vivo*, showing that pioglitazone inhibits pressure overload-induced cardiac hypertrophy strongly in wild type mice, and moderately so in heterozygous PPAR-γ deficient mice [406]. These pioglitazone-related effects on pressure-overload induced cardiac hypertrophy were associated with a significant reduction in the expression of endothelin-1 mRNA [408]. Endothelin-1 has positive inotropic and chronotropic actions, and induces cardiac hypertrophy [409]. In a murine study investigating effects at a pathophysiological level, Tsuji et al. showed that a pioglitazone induced reduction in left ventricular weight to body weight ratio was accompanied by a reduction in left ventricular collagen content, left ventricular diastolic dysfunction and plasma malondialdehyde-thiobarbituric acid (a marker of oxidative stress) [410]. Studies on intact animal models showed that PPAR-γ agonists improve cardiac contractility, systolic performance [411-414] and diastolic performance [412-415].

Myocardial intracellular calcium concentrations increase in response to myocyte stretch, in a bid to enhance cardiac output. The calcium, calmodulin-dependent phosphatase calcineurin plays a critical role in this process, through dephosphorylation of a family of transcription factors known as nuclear factors of activated T cells (NFATs) [416]. Four calcineurin sensitive NFAT isoforms have been identified (NFATc1, NFATc2, NFATc3, NFATc4) [417, 418]. Dephosphorylated by calcineurin, NFAT transcription factors translocate to the nucleus and regulate the expression of target myocardial genes [416, 419]. Activation of calcineurin or NFATc4 was shown to induce cardiac hypertrophy and HF in murine models [416]. Treatment of cardiomyocytes with rosiglitazone inhibited endothelin-1 induced calcineurin activity, enhanced the association of PPAR-γ with calcineurin/NFATc4 and suppressed the nuclear translocation of NFATc4 [420]. This observation is consistent with genotypic observations from the DREAM study, in which one single nucleotide polymorphism (SNP) in NFATC2 (rs6123045) was significantly associated with oedema [OR 1.89 (95% CI 1.47, 2.42) [421]. The effect is seemingly additive, with oedema rates being highest among patients homozygous for the risk allele, intermediate in heterozygous individuals, and lowest among subjects homozygous for the protective allele [421].

Multiple human studies have demonstrated no untoward effects on various parameters of cardiac performance and some trends toward improved systolic function associated with longer-term thiazolidinedione therapy. Ghazzi et al. compared echocardiographic data before and 48 weeks after randomizing 154 T2DM patients to treatment with troglitazone or glyburide, showing that thiazolidinedione therapy was associated with significant improvements from baseline in stroke

volume index and cardiac index, with no change in left ventricular mass index. There were no significant changes in any echocardiographic parameter in the glyburide-treated group [411]. A similar randomized, blinded clinical trial that included 203 patients did not report any significant differences in left ventricular mass index, ejection fraction or left ventricular end-diastolic volume between patients randomized to rosiglitazone versus glyburide [422]. A smaller study by Hirayama and co-workers investigated echocardiographic parameters in 10 male hypertensive T2DM men and 12 normotensive T2DM men treated with pioglitazone for 6 months. There was no change in fractional shortening (a simple way of measuring ejection fraction) in either group. Pioglitazone was however associated with a significant reduction in left ventricular mass in the normotensive group [423]. In a 52 week placebo controlled study of 224 T2DM patients with NYHA class I/II HF, rosiglitazone was not associated with any changes in left ventricular volumes, left ventricular ejection fraction or cardiac index [424]. Similarly, albeit a different study population, Horio et al. did not report changes in the absolute values of left atrial or left ventricular end-diastolic diameter in 30 non-diabetic patients with essential hypertension treated with pioglitazone for 6 months [425].

The cardiac antihypertrophic properties of exogenous PPAR-γ agonist therapy discussed above contrast with the results of other studies. Duan et al. reported that rosiglitazone treatment caused cardiac hypertrophy in wild type and cardiac-specific PPAR-γ knockout mice, suggesting that although PPAR-γ is essential for normal cardiac development, treatment with exogenous PPAR-γ agonists might be detrimental [405]. This raised the hypothesis that exogenous PPAR-γ-associated cardiac hypertrophy could reflect the anabolic consequences of improved insulin

sensitivity. However, comparing outcomes in wild type mice and mice with cardiomyocyte restricted knockout of insulin receptors, the non-thiazolidinedione PPAR-γ agonist 2-(2-(4-phenoxy-2-propylphenoxy) ethyl) indole-5-acetic acid increased heart weights by 16% in the former group and 22% in the latter, and induced similar fold increases in the expression of hypertrophic markers such as α-skeletal actin, brain natriuretic peptide, and ANP in both type of mice [426]. These outcomes suggested that thiazolidinedione-induced myocardial hypertrophy occurs independently of insulin signalling [426]. Indeed, it is plausible to hypothesize that cardiac hypertrophy is a consequence of thiazolidinedione-induced water retention [427], in keeping with the observation that 2-(2-(4-phenoxy-2-propylphenoxy) ethyl) indole-5-acetic acid-treated mice had typical echocardiographic features of volume overload (increased left ventricular diastolic diameters and increased cardiac output) [426]. In a pilot study randomizing 30 T2DM patients inadequately controlled in metformin and sulphonylurea to treatment with pioglitazone or insulin glargine for 26 weeks, Dorkhan et al. reported that left ventricular end-diastolic volume increased by 11% and left atrial systolic volume increased by 17% in the pioglitazone group ($p < 0.05$ for the difference between pioglitazone- and insulin-treated groups). There were no differences in the change of ejection fraction or left ventricular mass between the randomization arms [428]. The reported increases in left atrial volume may be of particular clinical significance in thiazolidinedione-treated T2DM patients, given the observation that this echocardiographic parameter carries prognostic significance in a variety of cardiac disorders and in the general population [429]. Moreover, one case series suggested that T2DM patients with diastolic dysfunction are more prone to developing thiazolidinedione-induced HF [430]. Although most patients can tolerate a 6-8% plasma volume expansion occurring

subacutely after initiation of thiazolidinedione therapy [163, 164, 431], diabetic patients with impaired cardiac reserve may manifest signs and symptoms of CHF in this setting.

There is currently considerable interest in the concept of ‘cardiac lipotoxicity’, wherein triglycerides are deposited in cardiac myocytes [432, 433], particularly in the setting of obesity or absolute or relative leptin deficiency/resistance (a phenotype associated with T2DM). Intracardiac triglyceride content was reported to be higher in obese human subjects, and was associated with increased left ventricular mass and decreased septal wall thickening [434]. Intramyocardial accumulation of ectopic fatty acid results in cellular dysfunction and non-oxidative fatty acid metabolism, which increases traffic through the ceramide pathway [432], resulting in lipoapoptosis, impairing cardiac compliance and contractility [435]. Troglitazone was shown to reduce intra-cardiomyocyte lipid concentrations and prevent loss of myocardial contractile function in a Zucker rat model [433].

In summary, the effects of PPAR-γ agonists on cardiac performance remain unclear, particularly given the conflicting results from animal studies. Human studies have been reassuring in this regard, albeit limited by the number of recruited patients and duration of follow-up. Further mechanistic research based on careful phenotyping is clearly warranted to clarify these issues further, particularly given the ongoing lack of large scale, prospective trials.

1.13 Other suggested ‘fluid-retaining’ mechanisms

Thiazolidinedione-induced oedema has also been ascribed to altered water-ion transport in the gastrointestinal tract. The latter hypothesis is borne out of the results of a study investigating the effects of troglitazone on rat and human duodenal mucosa cells. In this study, Hosokawa et al. demonstrated inhibition of electrogenic bicarbonate secretion by these cells, possibly interfering with passive sodium and water movement into the gastrointestinal tract lumen via a paracellular pathway [436].

1.14 Thiazolidinediones and heart failure: unanswered questions

Guided by the data summrised above, the manufacturers of rosiglitazone and pioglitazone excluded diabetic patients with New York Heart Association (NYHA) functional class III or IV HF from their applications for marketing licenses [163, 164]. The NICE guidelines have adopted a more stringent approach, and do not recommend the use of these drugs in any patient with HF, irrespective of severity [82]. While this approach is likely to minimize the risk of fluid retention, it does not eliminate it completely. Although thiazolidinedione-associated oedema is a clinically important adverse effect, absolute rates are low and the time course is uncertain. Traditional observational study designs have encountered difficulties in finding informative cases. Given the hypothesis that some individuals are more sensitive to the phenotype than others, it seems appropriate (and cost-effective) to study previously-intolerant individuals in some depth. The most appropriate comparator group is patients who are tolerant of thiazolidinediones.

Based on a case-control design, one of the aims of this thesis was therefore to identify and compare the baseline characteristics of matched cohorts of patients tolerant and previously intolerant of thiazolidinediones with the aim of assessing whether patients known to be intolerant to thiazolidinediones are characterised during acute or chronic 'high salt' loading by differences in their metabolic, cardiovascular and renal responses.

Given the anticipated difficulties in identifying thiazolidinedione intolerant patients, a parallel project was conceived in collaboration with the Health Informatics Centre (HIC) at the University of Dundee to identify and characterize NHS patients exposed to thiazolidinediones in routine clinical care. Prescription and hospital admission data were used to identify thiazolidinedione-treated patients whose treatment was apparently complicated by HF. Moreover, the Diabetes Audit and Research in Tayside Scotland (GoDARTS) cohort [437], a well-validated electronic data linkage – based register of diabetes patients in Tayside, Scotland enabled genetic characterization of the phenotypic characteristics identified at population level. At the time these studies were conceived, it was intended that they would provide sufficient background data to embark on a large prospective trial validating the usefulness of one or more biomarkers in the prediction of thiazolidinedione-associated fluid retention. Better characterization of thiazolidinedione intolerant patients, together with an adequate understanding of the mechanisms underlying thiazolidinedione-associated fluid retention was intended to guide the development and future assessment of PPAR-γ receptor modulators (and related agents such as dual PPARγ/δ agonists) with apparently more favourable adverse event profiles.

.*Section IV* - Insulin resistance in type 1 diabetes - is there a role for metformin?

1.15 Insulin resistance – a common co-morbidity in type 1 diabetes

While insulin resistance is undoubtedly a central pathognomonic feature of T2DM, its association with T1DM is also increasingly recognized. Insulin resistant patients with T1DM have been shown to express lower tissue levels of the insulin receptor [438]. Moreover, the expression of the GLUT-4 transporter in skeletal muscle is lower in obese patients with T1DM [439]. Insulin-insensitive patients with T1DM and T2DM have been reported to have raised intramyocellular lipid [440], which as discussed, interferes with insulin signalling. Comparing adipocytokine levels in 91 T1DM and 91 healthy children, Celi et al. reported that circulating adiponectin levels were higher in prepubertal diabetic children and positively correlated with HbA1c, while BMI-adjusted leptin concentrations were higher in pubertal diabetic children and positively correlated with daily insulin dose. There were no differences in TNF-α concentrations between the two groups [441]. Luna et al. similarly reported that children with T1DM were characterized by higher leptin concentrations compared with their healthy counterparts, but did not associate this finding with HbA1c, daily insulin dose or duration of the disease [442]. Two small studies investigating the kinetic mechanisms of insulin resistance in T1DM reported that impaired insulin-stimulated vasodilation impairs glucose delivery, and hence extraction at the level of skeletal muscle [443, 444].

Additional factors are thought to contribute to insulin resistance in pubertal T1DM patients. Insulin resistance increases in puberty, and reaches a peak at Tanner stage 3 [445]. The situation is further compounded by the fact that obesity is a growing problem in young patients with T1DM. Indeed, Libman et al. [446] showed that 50% of young Americans with T1DM were overweight or obese. Produced in the liver, insulin growth factor (IGF-1) plays a insulin-like role in glucose homeostasis, influencing hepatic glucose output and peripheral glucose uptake. Circulating IGF-1 levels are reduced in T1DM [447], possibly secondary to portal hypoinsulinaemia [448]. This results in a compensatory increase in Growth Hormone (GH) secretion (which antagonizes the effects of insulin) and IGF-1 binding protein synthesis [448, 449], diminishing free (and hence biologically active) IGF-1 levels further.

1.15.1 The 'accelerator' hypothesis

Although the functional effects of these multi-level differences are yet to be clearly elucidated, they have the potential to contribute to the mechanism of insulin resistance in T1DM individuals. It has been noted that T1DM is increasing in incidence and generally presenting at a younger age. Moreover, a smaller proportion of newly diagnosed patients are characterised by high risk and protective HLA haplotypes [450, 451], while a larger number have intermediate genetic susceptibility [452]. This suggests an increasing role of the environment in the aetiology of T1DM. Some [446, 453, 454], though not all studies [455, 456], have reported that obese patients with T1DM present at a younger age, possibly reflecting genetic and ethnic differences between study populations [457]. Additionally, BMI may have been too crude a measure of insulin resistance in pubertal patients [445]. Four studies carried

out in different continents analyzed data from prospective follow-up studies of autoantibody positive first degree relatives of patients with T1DM. Using the homeostasis model of insulin resistance (HOMA-IR) to first phase insulin response (FPIR) as an index that standardizes insulin resistance to residual β-cell function and corrects for falling FPIR as the T1DM process progresses, all four studies reported that insulin resistance is an independent risk factor for the development of T1DM [458-461]. Insulin resistance in pre-T1DM may not genetically determined, as suggested in a twin study by Hawa et al., in which patients developing T1DM had higher fasting insulin levels compared to their monozygotic twin counterpart who did not progress to the disease after 18 months of follow-up [462]. The hyperbolic insulin secretory response of the β-cells is dependent on normal insulin sensitivity [7]. Given that several studies have reported that islet-cell antibody individuals who progress to T1DM have greater insulin resistance for their level of insulin secretion [458-461], the insulin resistant state may unmask β-cell deficiency at an earlier stage [456, 463]. Adipocytokine receptors are expressed on the surface of immune cells [464], an observation that is likely to be relevant in the aetiopathogenesis of T1DM given that the intraperitoneal injection of leptin accelerated the autoimmune destruction of insulin-producing β cells and significantly increased interferon-γ production in peripheral T-cells in the non-obese diabetic mouse (a model of T1DM) [465]. Reports that insulin resistance is associated with a lower frequency of entering the ‘honeymoon phase’ in T1DM [466, 467] follow the same line of thought, potentially further justifying pharmacological attempts at reducing insulin resistance in T1DM. Thus, in summary, the accelerator hypothesis suggests that, while T1DM is essentially triggered by an immune mediated process, its progression is expedited by potentially modifiable factors such as insulin resistance and BMI [468]. This

essentially implies that, in the absence of a triggering immunological event, such patients would have developed T2DM at some point in their lives.

1.15.2 The concept of 'double diabetes'

The term 'double diabetes' was coined from the observation that patients with T1DM and a family history of T2DM were more likely to be overweight, required higher insulin doses and yet were less likely to achieve adequate glycaemic control [469]. This hypothesis considers T1DM and insulin resistance/obesity as independent processes. A study of 427 patients with T1DM reported that 15% fulfilled the WHO criteria for the metabolic syndrome, and of these 26.9% were insulin resistant, compared with 3.4% of those without metabolic syndrome (OR 8.9; p = 0.001). Those with the metabolic syndrome required higher median insulin dosage [0.9 (interquartile range = 0.7, 1.2) vs 0.6 (interquartile range = 0.5, 0.9) U/kg; p = 0.03], were older [median 35.0 (interquatile range = 26.2, 47.3) vs. 29.7 (interquartile range = 23.4, 36.4) years, p = 0.002], and had longer duration of diabetes [median 19.7 (interquartile range = 10.7, 25.6) vs. 12.1 (interquartile range = 6.3, 17.9) years, p = 0.0001] [23]. 21% and 44% of patients with T1DM met WHO diagnostic criteria of the metabolic syndrome in the Pittsburgh EDC [470] and FiannDiane [471] cohorts respectively. A parental history of hypertension has been associated with albuminuria in both men and women with T1DM. Additionally, albuminuria in women with T1DM was associated with parental diabetes in a cross-sectional study of 3250 patients recruited into the EURODIAB study [472]. Analyzing data from the Pittsburgh Epidemiology of Diabetes Complications Study, Erbey et al. concluded that T1DM patients with a first degree family member with T2DM were at higher

risk of coronary artery disease [OR 1.89 (95% CI 1.27, 2.84)]. However, this effect did not remain significant after adjusting for T1DM duration, triglycerides, hypertension, Beck depression and nephropathy status [OR 1.45 (95% CI 0.87, 2.28)] [473]. Nonetheless, a T1DM patient's risk of developing coronary artery disease increased with an increasing number of first degree family members suffering from T2DM (p = 0.001 for trend), such that the presence of two, rather than one family member virtually increased a T1DM individual's OR from 1.62 to 5.13 [473].

1.16 Consequences of insulin resistance in type 1 diabetes

McGill et al. reported that patients with T1DM and features of the metabolic syndrome were characterised by a higher macrovascular composite endpoint (OR = 3.3, p = 0.02), and a higher combined macrovascular and microvascular endpoint (OR = 3.1, p = 0.0001). Subdividing individuals with T1DM into duration of diabetes quartiles, the same investigators additionally reported that individuals diagnosed 20 or more years earlier and fulfilling the criteria for the metabolic syndrome were at a higher risk of stroke (OR = 22.8, p = 0.008) and severe retinopathy (OR = 3.7, p = 0.01); the risk of peripheral vascular disease was borderline (OR = 7.3, p = 0.05) [23]. Investigating 1337 Caucasian patients with T1DM fulfilling IDF diagnostic criteria for the metabolic syndrome and participating in the DCCT trial, Kilpatrick et al. [24] reported that insulin sensitivity (measured as estimated glucose disposal rate [eGDR] in mg/kg/min) strongly protected against the development of retinopathy (HR 0.75 per mg/kg/min; p < 0.001), nephropathy (HR 0.88 per mg/kg/min; p = 0.005) and cardiovascular disease (HR 0.70 per mg/kg/min;

p = 0.002). The authors also reported that the prevalence of the metabolic syndrome in these patients increased from 15.5% at baseline to 27.2% at year nine in conventionally-treated patients. The corresponding rise was higher in intensively-treated individuals (13.7% to 45.4%). These changes were attributed to weight gain [24].

Similar associations between markers of the metabolic syndrome, insulin resistance and individual macro-/micro-vascular complications in T1DM were reported in other studies [25-32]. A historical prospective cohort study of 603 patients with T1DM recruited from the Pittsburgh epidemiology of diabetes complications study and followed up for 10 years (excluding individuals with prevalent coronary artery disease) showed that insulin resistance, (measured using eGDR, and comparing lowest quintile versus the rest), predicted hard coronary artery disease endpoints (myocardial infarction, coronary artery disease death or angiographically proven stenosis) [HR 2.7 (95% CI 1.3, 5.6); p = 0.007 (from Cox proportional hazards model)] [27]. In general, this result is in agreement with that published by Soedamah-Muthu et al., who reported a relationship between waist-hip ratio (a surrogate measure of insulin resistance) and coronary heart disease in men participating in the EURODIAB Prospective Complications Study (n = 2329 patients) [HR 1.32 (95% CI 1.08, 1.62); p < 0.01 (from Cox proportional hazards model)] [28].

Olson et al. investigated the relationship between insulin resistance and peripheral artery disease in a cohort of patients from the Pittsburgh epidemiology of diabetes complications study (n = 586 patients), concluding that eGDR predicts the

development of lower extremity arterial disease (defined as claudication, foot ulceration or lower extremity claudication) in women [HR 0.45 (95% CI 0.32, 0.64); $p < 0.001$ (from Cox proportional hazards model)] [32].

A review of the relationship between insulin resistance and microvascular complications also yields significant data. In a separate publication based on follow-up data of patients from the Pittsburgh epidemiology of diabetes complications study (n = 485 patients), insulin resistance was reported to be a predictive factor for overt nephropathy in T1DM, both in the short term (1-5 years of follow-up) and in the long term (6-10 years of follow-up) [$p < 0.001$ for both associations (from Cox proportional hazards model)] [31]. Giorgino et al. analyzed data from 352 microalbuminuric patients with T1DM from 31 European centres recruited in the EURODIAB Prospective Diabetes Study. The investigators compared risk factors at baseline between patients who remained microalbuminuric, progressed to macroalbuminuria or reverted to normoalbuminuria. Baseline body weight was associated with progression to macroalbuminuria [Standardised estimate of relative risk (SERR) 1.5 (95% CI 1.1, 2.3); $p = 0.03$], together with HbA1c [SERR 2.1 (95% CI 1.4, 3.0); $p = 0.0003$] and albumin excretion rate [SERR 1.9 (95% CI 1.3, 2.8); $p = 0.0006$]. [30]. de Boer et al. investigated whether waist circumference is associated with incident microalbuminuria and a change in creatinine clearance among 1279 patients with T1DM who were enrolled in the Diabetes Control and Complications Trial (DCCT). Each 10 cm increase in waist circumference was associated with a significantly increased risk of incident microalbuminuria [HR 1.34 (95% CI 1.07, 1.58)], after adjusting for age, gender, race, duration of diabetes, treatment group,

smoking status, waist circumference, HbA1c and albumin excretion rate (each of which were measured at DCCT close-out) [25].

Analyzing data from 764 patients with T1DM recruited into the EURODIAB study and followed up for 7.3 years, Chaturvedi et al. reported that waist-hip ratio is a risk factor for developing retinopathy [standardized regression estimate (SRE) 1.32 (95% CI 1.07, 1.63); p = 0.01], together with duration of diabetes [SRE 1.32 (95% CI 1.07, 1.61); p = 0.008], HbA1c [SRE 1.93 (95% CI 1.52, 2.44); p = 0.0001] and fasting triglyceride levels [SRE 1.24 (95% CI 1.01, 1.54); p = 0.04] [26].

Data from the EURODIAB study was also used to investigate the association between insulin resistance, its surrogate measures and incident distal symmetric neuropathy in 1172 patients with T1DM. Adjusting for HbA1c values and duration of diabetes, weight [OR 1.34 (95% CI 1.17, 1.54); p < 0.001], BMI [OR 1.40 (95% CI 1.22, 1.61); p < 0.001] and a lower eGDR [OR 1.37 (95% CI 1.08, 1.73); p = 0.01] were associated with an increased risk of incident neuropathy [29].

1.17 Is there a conceptual role for metformin in type 1 diabetes?

Intensive glycaemic control in patients with T1DM was reported to decrease the long term risk of cardiovascular disease by 42% and the risk of nonfatal myocardial infarction, stroke or cardiovascular death by 57% [474], with changes in HbA1c (rather than changes in cardiovascular risk factors) seemingly accounting for most of the benefit. However, the DCCT showed that intensive insulin therapy is hampered by excessive weight gain, resulting in visceral adiposity [475], and deletirious effects

on lipids, blood pressure [476] and inflammatory markers [477]. Similar findings were reported in the EURODIAB study, with T1DM patients gaining more than 5 kg over a mean observation period of 7.3 years being characterised by better glycaemic control at the expense of higher blood pressure, LDL-cholesterol and triacylglycerol, and lower HDL-cholesterol [478]. Subcutaneous insulin administration is associated with relative peripheral hyperinsulinaemia and relative hepatic hypoinsulinaemia [479]. Surprisingly, although portal administration of insulin increased IGF-1 and reduced prevalent GH levels, this occurred at the expense of a more atherogenic lipid profile (reduced HDL-cholesterol, increased LDL-cholesterol:HDL-cholesterol ratio) [480-484]. Whether this effect translates into a adverse cardiovascular outcomes remains to be determined. Despite relative hepatic hypoinsulinaemia, glucagon secretion is preserved in T1DM, favouring lipid oxidation. Exogeneous subcutaneous insulin administration further enhances this process, increasing circulating levels of non-esterified fatty acids and fuelling lipid accumulation in skeletal muscle [440, 479, 485, 486].

These findings may justify the addition of the weight neutral insulin-sensitizing drug metformin, the use of which (in T2DM) has been associated with modest reductions in serum triacylglycerol, VLDL and LDL levels, decreased C reactive protein, decreased platelet activation and a reduction in procoagulant factors (such as factor VII and fibrinogen) [487].

1.18 Metformin in type 2 diabetes - benefits beyond glycaemic control

The United Kingdom Prospective Diabetes Study (UKPDS) was the first major study underpinning the cardiovascular benefits of metformin in T2DM. The study randomized 1704 overweight patients with T2DM to initial treatment with metformin (342 patients), sulphonylurea/insulin (951 patients) or dietary measures alone (411 patients). Compared with dietary measures, metformin (but not sulphonylurea/insulin therapy) was associated with a 32% lower incidence of any diabetes-related endpoint (micro and macrovascular) (95% CI 13, 47; p = 0.002), 42% fewer diabetes related deaths (95% CI 9, 63; p = 0.017), 36% lower all-cause mortality (95% CI 9, 55; p = 0.011), and 39% fewer myocardial infarctions (MIs) (p = 0.010) [488]. These effects persisted after 10 years of follow-up [risk reductions of 21% for any diabetes related end-point (p = 0.01), 33% for myocardial infarction (p = 0.005), and 27% for death from any cause (p = 0.002)], despite the fact that differences in glycaemic control (as assessed by HbA1c levels) were lost after one year of follow-up [489].

The cardiovascular benefits of metformin in high risk patients with T2DM was elucidated by the the Prevention of Restenosis with Tranilast and its Outcomes (PRESTO) trial. This double-blind randomised controlled trial compared cardiovascular outcomes in patients treated with tranilast following a percutanouse coronary intervention. After 9 months of follow-up, patients treated with metformin (with or without additional therapy, n = 887) were at a significantly lower risk of death [OR 0.39 (95% CI 0.19, 0.77); p = 0.007) and myocardial infarction [OR 0.31

(95% CI 0.15, 0.66); p = 0.002] compared to those treated with insulin and/or sulphonylurea (n = 1110) [490].

Similar beneficial outcomes were noted following retrospective analysis of data from several large databases of patients with T2DM. The Diabetes and Audit in Research Tayside Scotland (DARTS) study (n = 5730 patients) reported that mortality was significantly lower after 5 years among drug-naïve T2DM patients initially treated with metformin compared to a sulphonylurea [491]. McAfee et al. compared cardiovascular outcomes in T2DM patients commenced on metformin, a sulphonylurea or rosiglitazone over a period of 4.5 years and whose data were extracted from a large US insurance database (n = 33363). Metformin monotherapy was associated with a lower risk of the composite endpoint of myocardial infarction and coronary revascularization after 5 years of follow-up compared to sulphonylurea monotherapy [HR 0.77 (95% CI 0.62, 0.96)] [492]. These findings are consistent with the results of other studies. Eurich et al. analyzed data of 12,272 new users of oral hypoglycaemic agents suffering from T2DM and HF, recruited from the Saskatchewan Health Database (n = 1833, average age 72 years) and followed up for a mean of 2.5 years. Metformin monotherapy was associated with a lower risk of mortality [HR 0.70 (95% CI 0.54, 0.91)] and a lower risk of the composite outcome of deaths or hospitalizations [HR 0.83 (95% CI 0.70, 0.99)] compared to sulphonylurea monotherapy [281]. A retrospective study of 16417 Medicare beneficiaries with HF discharged after hospitalization with a principal diagnosis of HF showed that metformin pharmacotherapy was associated with a reduction in crude 1-year mortality rates (24.7% vs 36.0% of patients not treated with an insulin sensitizing drug; p < 0.0001), a result confirmed in multivariate analysis [HR 0.87

(95% CI 0.78, 0.97)] [283]. Metformin was also associated with a modestly reduced risk of readmission with HF [HR 0.92 (95% CI 0.86, 0.99)] but no effect on all-cause readmissions [283]. A systematic review and meta-analysis of eight controlled studies compared outcomes between different antihyperglycaemic agents in T2DM patients with HF [284]. Metformin was associated with significantly reduced all cause mortality in two studies [HR 0.86 (95% CI 0.78, 0.97)] (n = 1861 patients), and with similar trends in a third study, compared with non-sensitisers (sulphonylureas, non-sulphonylurea insulin secretagogues, alpha glucosidase inhibitors or insulin) (n = 12069 patients). Formal meta-analysis showed that metformin was not associated with any significant effects on all-cause hospitalization, albeit a lower risk for heart-failure related readmissions [HR 0.92 (95% CI 0.86, 0.99)] [284], in keeping with the findings of Masoudi et al. [283].

Of potential direct relevance to T1DM, Kooy et al. compared outcomes in 390 insulin-treated patients with T2DM randomized to treatment with metformin or placebo therapy and followed up for 4.3 years [493]. Adjunct metformin pharmacotherapy was associated with a reductions in body weight [-3.07 kg (range -3.85 to -2.28); $p < 0.001$], HbA1c level [mean -0.4% (95% CI -0.25, -0.55); $p < 0.001$] and insulin requirements [mean -19.63 IU/day (95% CI -14.36, -24.91); $p < 0.001$]. Additionally, metformin was reported to decrease macrovascular morbidity and mortality (HR 0.61 [95% CI 0.40, 0.94]; $p = 0.02$), an effect that was partly explained by the difference in weight [493].

In a retrospective study on 8063 patients with no prior history of congestive HF, Nichols et al. compared the incidence of HF between individuals who were

commenced on additional treatment for T2DM over a period of 4 years [494]. The prescription of metformin to insulin-treated patients with T2DM reduced the congestive HF rate ratio to 0.63 (95% CI 0.3, 1.07), a development which is particularly desirable given that initial insulin therapy was associated with a higher incidence of congestive HF [44.5/1000 patients/year (95% CI 37.9, 52.3)] than metformin [15.3/1000 patients/year (95% CI 8.9, 26.3)] or sulphonylureas [19.9/1000 patients/year (95% CI 17.2, 23.1)] [494]. In a similar vein, the systematic review by Eurich et al. had also reported that insulin treatment was associated with increased cardiovascular morbidity (hospital admission for HF, prescription for open label angiotensin converting enzyme inhibitor, or myocardial infarction) and mortality [HR 1.38 (95% CI 1.06, 1.80)] [284].

1.19 Use of adjunct metformin in type 1 diabetes: what is the evidence?

As suggested above, the available data underpinning the use of metformin in T2DM justify examining the safety and efficacy of this drug in T1DM. Its low cost, proven safety profile and promising short and long-term macro- and microvascular benefits in T2DM justify studies to define its use in a disease increasingly associated with insulin resistance and other components of the metabolic syndrome. This thesis will therefore examine the available evidence supporting the use of this drug in T1DM, and investigate the hypothesis that adjunct metformin is associated with (i) a decrease in insulin dose and weight, and (ii) an improvement in glycaemic control and lipid profile.

Chapter 2

Clinical study

Characterising thiazolidinedione 'tolerant' and 'intolerant' patients

A physiological approach

Chapter 2 - Clinical study

Characterising thiazolidinedione 'tolerant' and 'intolerant' patients

A physiological approach

Section I - Methods

2.1 Study design

This clinical study was a case-control biomarker study in T2DM patients aged 40 to 70 years. It compared physiological parameters between thiazolidinedione 'tolerant' and 'intolerant' patients in response to a low, acute high and chronic high salt diet. This was achieved during three study visits.

2.2 Good clinical practice

The study was conducted in accordance with the protocol, protocol amendments, Good Clinical Practice (GCP) as described in the International Conference on Harmonization (ICH) tripartite harmonized guidelines of technical requirements for registration of pharmaceuticals for human use [495], the Declaration of Helsinki (2000 Edinburgh) [496], the ‘Research Governance Framework for Health and Community Care’, second edition, 2006 [497], and applicable legal and regulatory requirements

2.3 Ethics

In accordance with the above guidelines governing medical research, the study protocol and its subsequent amendments were subjected to ethical approval by the Tayside Research Ethics committee. The protocol for this study was approved in February 2008. A number of amendments pertaining to study documentation were subsequently submitted for ethical approval in view of difficulties with patient recruitment, funding withdrawal by Wyeth Pharmaceuticals, and a re-location of the principal investigator. Patient recruitment and study procedures were allowed to commence and/or proceed only when the ethics committee approval letter had been received by the Principal Investigator at each stage. Protocol amendments were prepared by the undersigned, working as a Clinical Research Fellow in this project, and approved by the Principal Investigator. Administrative amendments that did not affect the conduct of the study or patient safety, and did not significantly reduce the scientific value of the protocol did not require a formal review and approval from the Ethics Committee. A copy of all correspondence between the investigator and the Ethics Committee was kept in the appropriate section of the study file. The Clinical Research Fellow or Principal Investigator was bound to follow local institutional guidelines on reporting serious adverse events.

2.4 Caldicott-Guardian approval

ICH GCP section 2.13 states that ‘systems with procedures that assure the quality of every aspect of the trial should be implemented’ [495]. The identification of potentially suitable patients was aided by access to SCI-DC datasets. With this in

mind, the data collection process was subjected to Caldicott-Guardian approval in accordance with established protocols [498], and only commenced once the necessary approval letter was received by the Principal Investigator. A copy of all study-related correspondence pertaining to Caldicott-Guardian approval was kept at the appropriate section of the study file.

2.5 Study objectives

2.5.1 Objective 1 – to compare the baseline characteristics of two cohorts of patients with type 2 diabetes who are either sensitive or insensitive to thiazolidinedione-associated fluid retention

Although thiazolidinedione-induced oedema is a clinically important adverse effect, absolute rates are low and the time course is uncertain. Traditional observational studies have encountered difficulties in finding informative cases. Given the hypothesis that some individuals may be more prone to thiazolidinedione-associated oedema than others, it seemed appropriate and cost-effective to study these 'intolerant' individuals in some depth. The most appropriate comparator group was a cohort of thiazolidinedione 'tolerant' patients. The potentially confounding effect of thiazolidinedione therapy was avoided by substituting these antihyperglycaemic agents with sulphonylurea therapy for 4 weeks prior to the study interventions, while maintaining stable glycaemic control.

2.5.2 Objective 2 – to compare the characteristics of the above two cohorts during an acute and chronic 'high normal' sodium loading in order to detect differences in metabolic, cardiovascular and renal characteristics.

This case-control study, comparing cohorts of matched thiazolidinedione 'tolerant' and previously 'intolerant' patients, was designed to address the following hypotheses:

Primary hypotheses:

Are patients known previously to have been intolerant of thiazolidinediones characterised during either acute or chronic "high normal" sodium loading by:

1) Increased ankle-foot volume (AFV) (a measure of oedema)

2) Impaired left ventricular diastolic function (including tissue Doppler)

3) High pulse wave velocity

4) Salt sensitivity of blood pressure

5) High plasma VEGF levels

Secondary hypotheses:

Do renin-angiotensin system activation, fractional sodium excretion, free water handling and/or total body water (deuterium dilution) differ between cohorts 1 and 2 during acute or chronic sodium loading?

2.6 Study population

The study recruited male and female T2DM patients aged between 40 and 70 years of age with a history of thiazolidinedione exposure. These patients were subdivided into two cohorts:

- A thiazolidinedione 'tolerant' cohort (cohort 1) defined by T2DM individuals previously initiated on thiazolidinediones (usually, but not exclusively in combination with metformin), with HbA1c ≤9.0% and without diuretic therapy, whose current thiazolidinedione therapy was not complicated by fluid retention and/or HF.

- A thiazolidinedione 'intolerant' cohort (cohort 2) defined by T2DM patients whose thiazolidinedione therapy was withdrawn within three months of onset of thiazolidinedione exposure as a consequence of drug-associated fluid retention and/or HF, and now with an HbA1c of ≤9.0% on one or two non-thiazolidinedione agents (sulphonylreas/metformin) and without diuretic therapy.

The cohorts were matched as far as possible for age and gender. Only patients who met all inclusion criteria and no exclusion criteria were considered for participation in this study.

2.7 Inclusion criteria

All patients recruited into the study were required to fulfil all of the following criteria:

- Adult patients tolerant (cohort 1) or previously-intolerant (cohort 2) of thiazolidinediones
- T2DM
- Aged ≥40 years and ≤70 years
- Recorded HbA1c ≤9.0% within last six weeks
- Non-microalbuminuric (either negative single morning sample or tested at screening)
- Recorded blood pressure ≤145/85 mmHg on no therapy, monotherapy or dual therapy
- Ability to understand and willingness to sign the informed consent form

Patients in Cohort 1 (tolerant of thiazolidinediones) were additionally required to fulfil all of the following criteria:

- Previously initiated and currently continuing on thiazolidinedione therapy without diuretic therapy
- Prepared to discontinue thiazolidinedione therapy with informed consent
- Prepared to take an alternative treatment instead of thiazolidinedione therapy

Patients in cohort 2 (previously intolerant of thiazolidinediones) were likewise additionally required to fulfil all of the following inclusion criteria:

- Previously withdrawn from thiazolidinedione therapy at any stage because of reported fluid retention (including oedema and/or HF)
- Currently being treated with one or two non-thiazolidinedione oral anti-hyperglycaemic agents
- No current diuretic therapy

2.8 Exclusion criteria

Patients were excluded from the study if they met any of the following criteria:

- BMI > 40 kg/m^2
- HbA1c > 9.0%
- Patients (Cohort 2) withdrawn from thiazolidinedione therapy for reasons other than oedema (e.g. weight gain without oedema, liver dysfunction, lack of efficacy, other adverse events).
- Hypertension requiring treatment with three or more anti-hypertensive agents
- HF (NYHA Classes II, III, IV or left ventricular systolic ejection fraction < 40%)
- Significant renal or hepatic dysfunction (defined as a serum creatinine level exceeding 130 μmol/L or a > 2.5 fold increase in prevalent alanine aminotransferase (ALT) levels respectively)
- Known to be HIV-positive
- Known active hepatitis B and/or hepatitis C infection
- Pregnant or lactating women
- Known drug/alcohol abuse
- Known psychiatric condition

Both men and women and members of all ethnic groups were eligible for this study. Pregnant women were excluded for safety reasons. Children were not eligible – the study population encompassed individuals aged 18 – 70 years.

2.9 Withdrawal from the study

Patients had the right to withdraw from the study at any stage, for any or no specific reason, without affecting any of their statutory rights as patients or continuing care. The investigator had the right to withdraw patients in accordance with the following guidelines:

- At his discretion, if it was perceived to be in the best interests of the patient to withdraw
- Intercurrent illness: a condition, injury or disease unrelated to diabetes, that rendered continuing the study unsafe or regular follow-up impossible
- General or specific changes in the patient's condition that rendered the patient ineligible
- Noncompliance with study procedures or protocol-required evaluations
- Termination of the clinical study by the sponsor or funding body

The reasons for any withdrawal(s) were clearly explained in the case report form (CRF).

2.10 Recruitment process

The study recruited T2DM patients whose routine management is monitored within the Scottish Care Information – Diabetes Collaborative (SCI-DC) clinical information system in Tayside, Scotland. This confidential password-protected national computerised clinical system provides up-to-date single patient records, yielding a Scottish-wide register of all patients with diabetes based on a unique nine-digit patient identifying number [Community Health Identifying (CHI) number]. Primarily designed to deliver integrated diabetes care to all members of the diabetes care team, it is also an invaluable research tool for recruitment purposes [437]. The original Diabetes Audit and Research Tayside (DARTS) database for Tayside has a sensitivity of 96% and a positive predictive value of 95% for ascertainment of known diabetes [437]. At the time of the study, SCI-DC consisted of two separate elements, called SCI-DC clinical and SCI-DC network. The former was a secondary care clinical management system whilst the latter was a web-based clinical system containing data from primary and secondary care (both were superceded in 2012 by a single web-based system, SCI-Diabetes). SCI-DC data is linked to the Medicines Monitoring Unit (MEMO) database. The latter was developed for pharmacoepidemiological research in the population of Tayside, and contains detailed records of all prescription items dispensed to patients at community pharmacies [499]. Thus, at the time of the study, detailed records of all prescriptions dispensed for thiazolidinediones, insulin, diuretics, and all other drugs referred to hereafter were available for all Tayside patients (now across Scotland). This highly integrated clinical information system proved indispensable in identifying the two

groups of thiazolidinedione-treated patients fitting the very specific inclusion criteria for this study.

The method of approaching T2DM patients followed the Standard Operating Procedures developed by the Scottish School of Primary Care, formerly Scottish Practices and Professionals Involved in Research (SPPIRe) [500]. Thus, the University of Dundee Health Informatics Centre produced a computer diskette permitting the interrogation of the computer systems of participating practices by study research nurses. An algorithm was used to identify patients on thiazolidinedione therapy (cohort 1) or in receipt of up to three (but not more) previous prescriptions for rosiglitazone or pioglitazone in the last two years (cohort 2). Individual general practitioners were contacted regarding patients who were likely to fit the inclusion criteria. General practitioners who agreed were invited to forward a signed letter to the patient inviting them to participate in the study. This approach ensured that only patients who were likely to fit the inclusion criteria were actually contacted, minimizing patient inconvenience.

Additionally, the University of Dundee Health Informatics Centre identified potential patients who had been recruited into the Wellcome Trust Case Control Genetics study (LREC ref 053/04). These patients had consented to be re-contacted regarding participation in future research. Patients fulfilling the preliminary recruitment criteria (as assessed on the SCI-DC clinical information system) were written to using a standard letter specific to the cohort, accompanied by an information sheet outlining the nature of the study. Where telephone contact details were available in this Wellcome Trust dataset, this correspondence was followed up

by means of a phone call made by the author, a clinical research fellow in this study. An alternative recruitment approach was used in those instances where the patient's telephone number was not available from the study database. A letter of invitation was sent to these patients. This included a tear- off slip allowing invited patients to indicate whether they were interested in participation and to provide current contact details in the prepaid envelope provided. For patients who are willing to participate, screening of suitable patients was aided by clinical data available on the SCI-DC clinical information system. This recruitment approach commenced after obtaining Caldicott-Guardian approval.

The author personally invited participation by T2DM patients who were likely to fulfil the study inclusion criteria (as suggested by available SCI-DC records) when they attended the Diabetes Clinic at Ninewells Hospital between December 2008 and April 2010. This process commenced after obtaining the necessary Caldicott-Guardian approvals.

2.11 Study procedure - visit 1

Patients identified as potentially suitable either for inclusion into cohort 1 (thiazolidinedione tolerant) or cohort 2 (thiazolidinedione intolerant) were provided with one of two specific patient information sheet, outlining the aims and method of this study. They were also informed about the potential benefits and adverse effects associated with participation. The information sheet explained that participation, while greatly appreciated, was entirely voluntary. Patients were free to decline the invitation, or withdraw from participating at any stage. They were not obliged to

explain their reasons for doing so; additionally, it was clarified that such a decision did not adversely affect their statutory rights as patients. Subjects were offered the opportunity to clarify any concerns with the study research nurses, myself, the principal investigator or an external advisor (Professor Ewan Pearson). All patients considered for participation were recorded on a screening log that was maintained at the study site.

Patients expressing an interest were thus scheduled for a first study visit, which also encompassed a screening procedure. Transport was provided for all study and monitoring visits. In accordance with procedures approved by ethics committee, patients were compensated for time and inconvenience incurred as a result of participation in this study (£50 for each study visit), and for any travelling costs incurred if they opted to travel to the study site, as per ethical approval.

Patient participants were once again familiarised with the study schedule and given the opportunity to ask questions. I was delegated with responsibility for obtaining informed consenting using the approved form. The consent encompassed the extraction of routine clinical data from the SCI-DC system.

The following data were subsequently collected:

- Date of birth and age
- Gender
- A brief structured questionnaire detailing patient's experience of thiazolidinedione therapy, with particular emphasis of duration of thiazolidinedione therapy, ankle swelling, fluid retention, symptoms or signs

of HF, other adverse effects while on thiazolidinedione therapy (including hypoglycaemia)

- Diabetes related history with collection of data on diagnosis, macrovascular and microvascular complications. Status was assessed through available SCI-DC records. Retinal screening was repeated if not assessed within the last 12 months
- Past medical history
- Concomitant medications
- Weight was measured to the nearest 0.1 kilogram (kg) using an electronic, recently calibrated weighing scale. Patients were asked to stand unattended and barefoot on both feet at the centre of the weighing platform scale.
- Standing height was measured to the nearest 0.5 centimetre (cm) using a Leicester height measure. The subject was asked to remove any head dress or head ornaments and to stand barefoot with his/her back against the height rule, such that the back of the head, back, buttocks, calves and heels were touching the vertical scale, feet together. The attending clinical research fellow ensured that the patient was looking straight ahead, with the top of the external auditory meatus level with the inferior margin of the bony orbit. The apparatus' horizontal measure was lowered to touch the top of the head once the latter was correctly positioned.
- Waist circumference was measured using a flexible but non-stretchable measuring tape to the nearest 0.1 cm midway between the lower rib margin and the iliac crest at the mid-axillary line. The measuring points on each side were determined by marking these bony margins using a water-soluble marker pen, and determining and marking the midway point for each side.

Patients were asked to remove clothing from around the waist and hips. Measurement was taken with the subjects standing on both feet, with their feet pointing forwards and approximately 25-30 cm apart. They were asked to breathe normally. The reading of the measurement was taken at the end of a gentle exhalation. BMI was calculated by dividing each subject's weight in kilograms by the square of the height in metres

- Pregnancy test (if applicable) – patients with a positive pregnancy test were excluded
- Pulse and blood pressure were recorded in triplicate after 5 minutes of rest, in the non-dominant arm and sitting posture, using an automated sphygmomanometer placed at the level of the patient's heart and approved by the British Hypertension Society. Patients were asked not to cross their legs while sitting. They were also advised to refrain from smoking, drinking tea, coffee or cola, and participating in any arduous activity for one hour prior to blood pressure measurement. Adherence to these recommendations was verified at the study visit. Measurements were taken using an appropriately sized cuff that covered 80% of the circumference of the midpoint of the upper arm, after removing or loosing any clothing covering this site. Care was taken to ensure that the cuff was rotated such that the indicated mark on the cuff was placed over the brachial artery. The arm was rested on a pillow or bed while the measurement was being taken. Patients were asked to refrain from moving and speaking for a minute while the blood pressure was being recorded. Subjects were rested for five minutes before repeating the readings. All three pulse and blood pressure readings were recoded on the CRF, enabling the calculation of a mean reading for each clinical parameter.

Patients were excluded from participation if their mean blood pressure (on current antihypertensive therapy if applicable) exceeded 145/85mmHg.

- Physical examination of the cardiovascular, respiratory, abdominal, neurological, locomotor and endocrine systems was carried out.
- Venous blood samples were taken for estimation of full blood count, HbA1c, sodium, urea, creatinine, glucose, liver function tests and lipid profile.
- Dipstick urinalysis and urine for microalbuminuria – A second morning mid-stream urine sample was collected in a sterile universal container for this purpose. Patients were appropriately counselled by the research nurse or clinical research fellow prior to this procedure
- ECG

Cohort 1 patients were asked to replace their current thiazolidinedione therapy with gliclazide therapy at the same visit. They were provided with a glucose meter, and advised to check and record their blood glucose readings on the diary provided. The clinical research fellow maintained telephone contact with these patients, titrating sulphonylurea dose if necessary to maintain prevailing HbA1c at <9%. Cohort 2 (thiazolidinedione 'intolerant' patients were advised to continue therapy with their current antihyerglycaemic agents.

Individuals treated with (one or two) antihypertensive agents were advised to discontinue these agents, one at a time, with careful follow-up of blood pressure readings at each stage. Patients were withdrawn from the study if their blood pressure exceeded 160/110 mmHg following the withdrawal of one or both antihypertensive agents. Aspirin-treated individuals were advised to discontinue this

agent 10 days prior to visit 2. Patients were advised to recommence treatment with aspirin and antihypertensives once visit 3 was completed.

Participating individuals were asked to follow a moderate low (100 mmol/day) sodium diet for five days prior to visit 2; written information was provided in this regard. They were additionally supplied with self-weighing scales and urine specimen collecting containers enabling self-weighing and the collection of early spot urine samples for urinary sodium and urinary creatinine estimation for 5 consecutive days prior to the next visit.

2.12 - Study procedure - visit 2

Visit 2 was scheduled 4-7 weeks after visit 1, allowing adequate ‘wash-out’ of thiazolidinedione effects in thiazolidinedione 'tolerant' individuals (cohort 1). Patients were instructed to follow a moderately low (100 mmol/day) salt diet for five consecutive days prior to the study visit. On these days, participating subjects were asked to collect an early morning urine sample for urinary sodium and creatinine estimation, and to weigh themselves on awakening, dressed in their underwear, using the electronic self-weighing scale provided, recording the measurements on a diary. Patients arrived at the vascular research laboratory at around 08.30 hours. They were instructed to consume 300 mg lithium carbonate at 22.00 hrs the previous night and subsequently remain starved. Patients were asked to refrain from smoking and consuming alcohol and caffeine-containing beverages for the duration of the fast; compliance to this advice was verbally ascertained at the start of the study visit. On arrival, patients were made comfortable on a bed, and remained supine, except for

voiding and ankle-fluid measurements. Patients remained fasted until the end of the study visit, which lasted till about 16.00 hours. Patients were permitted to drink a volume of water equivalent to urinary losses throughout this visit. They were additionally provided with a sandwich meal before leaving the unit

2.12.1 Baseline measurements

During this study visit, the following baseline assessments and measurements were made:

- Echocardiography including tissue Doppler
- Concomitant therapy
- Compliance with diet
- Compliance with medication
- Assessment of occurrences of hypoglycaemia
- Dipstick urinalysis and urine for microalbuminuria
- Weight and waist measurement
- Blood pressure (in triplicate)
- AFV by water displacement
- Pulse wave analysis and velocity.
- HbA1c
- Plasma for biochemistry (urea and electrolytes, liver function tests)
- ANP
- Aldosterone
- Renin
- BNP and NT-proBNP

- VEGF
- AVP

2.12.2 Biochemistry

Blood samples for HbA1c estimation were collected in a vacuum collection tube containing EDTA. while plasma glucose samples were collected in a vacuum collection tube containing FX sodium fluoride/potassium oxalate. Sera for renal, liver and lipid profiles, serum albumin and lithium measurement were collected in a Z serum clot activator vacuum collection tube with gel separator. Details pertaining to assay methodology are outlined in table 2.1 below. Urinary lithium levels were measured by Mr Neil R Johnston in Professor David Webb's laboratory at the Clinical Pharmacology Unit, Queens' Medical Research Institute, University of Edinburgh, Edinburgh, using the flame photometry technique. The latter is characterized by an intra-assay CV of 1.54% and an inter-assay CV of 2.98%, based on repeated analysis of the control sample. The measuring instrument, a BWB-1 Flame Photometer (BWB Technologies) has a working range of between 1 and 100 ppm lithium. All other analyses were carried out at NHS Tayside laboratories, Ninewells Hospital and Medical School, Dundee.

Table 2.1 - Biochemistry assay methodology

Assay	*System*	*Method principle*
Serum sodium	Roche SWA	Indirect measuring ion-selective electrode
Serum potassium	Roche SWA	Iindirect measuring ion-selective electrode
Serum urea	Roche SWA[a]	Kinetic urease
Serum creatinine	Roche SWA[a]	Compensated kinetic Jaffe
Plasma glucose	Roche SWA[a]	Hexokinase
Bilirubin	Roche SWA[a]	Diazo
Serum alkaline phosphatase	Roche SWA[a]	IFCC
Serum alanine aminotransferase	Roche SWA[a]	IFCC without pyridoxal phosphate activation
Serum GGT	Roche SWA[a]	IFCC
Serum AST	Roche SWA[a]	IFCC without pyridoxal phosphate activation
Serum albumin	Roche SWA[a]	Bromocresol green
Serum total cholesterol	Roche SWA[a]	Cholesterol oxidase/peroxidase
Serum HDL-cholesterol	Roche SWA[a]	PEG-modified cholesterol esterase/PEG-modified cholesterol oxidase/peroxidase
Serum triglycerides	Roche SWA[a]	lipoprotein lipase/glycerokinase/glycerol phosphate oxidase/peroxidase
Glycosylated haemoglobin (HbA1c)	Menarini HA 8160	
Serum lithium	Roche AVL 9181	Direct measuring ion-selective electrode
Urine sodium	Roche SWA	Indirect measuring ion-selective electrode
Urine creatinine	Roche SWA[a]	Compensated kinetic Jaffe
Urine microalbumin	Roche Integra 800	Immunoturbidimetry
Urine lithium	BWB Technologies	Flame Photometer

[a] *P800 module*

Estimated glomerular filtration rate (eGFR) was derived using the abbreviated MDRD equation (GFR [mL/min/1.73m^2] = 175*serum creatinine in mg/dL$^{-1.154}$*age in years$^{-0.203}$*0.742 if female*1.212 if African American) [501]. Low density lipoprotein cholesterol (LDL-C) was indirectly derived from serum total cholesterol, high density lipoprotein cholesterol (HDL-C) and triglyceride values using the Friedewald formula [LDL-C = total cholesterol – HDL-C – (triglycerides/2.22)], all values being in mmol/L [502].

2.12.3 Biomarkers

Plasma samples for measurement of Vascular Endothelial Growth Factor (VEGF) and high sensitivity copeptin were assayed by Dr. Paul Welsh in the laboratory of Professor Naveed Sattar at the Institute of Cardiovascular and Medical Sciences, University of Glasgow. The high sensitivity copeptin assay used is characterised by a functional assay sensitivity of less than 2 pmol/L, an intra-assay CV of < 3% (for hs copeptin concentrations exceeding 50 pmol/L) to < 15 % (for hs copeptin concentrations of 3-4 pmol/L) and an inter-assay CV of < 5% (for hs copeptin concentrations exceeding 50 pmol/L) to < 17% (for hs copeptin levels of 3 to 4 pmol/L). The VEGF assay used gives a functional assay sensitivity of < 5 pg/mL, an intra-assay CV of 4.5% to 6.7% and an inter-assay CV of 6.2% to 8.8%. Blood samples for estimation of hs copeptin and VEGF concentrations were both collected in vacuum collection tubes containing EDTA, stored at -80 ºC until assay, thawed overnight in a refrigerator at 4 ºC, and mixed by inversion prior to assay. The rest of the biomarkers [ANP, B-type natriuretic peptide (BNP), N-terminal prohormone of brain natriuretic peptide (NT-pro-BNP), aldosterone and renin] were analysed by Ms. Leslie McFarlane at the laboratories of the Division of Cardiovascular and Research Medicine, Medical Research Institute, University of Dundee. Blood samples for ANP, BNP, and renin were collected in vacuum collection tubes containing EDTA, and immediately spun (3000 rpm) for 10 minutes at 4ºC. Two 2 mL plasma aliquots were collected in 5mL plastic tubes and frozen at - 70ºC until formal analysis of ANP and BNP levels. Two 1mL plasma aliquots were likewise collected in 1.5mL plastic tubes and frozen at -20ºC pending formal renin level estimation. Both ANP (reference range: 8.6 ± 0.8 pg/mL) and BNP (reference range:

3.9 ± 0.3 pg/mL) assays were characterised by intra- and inter-assay CVs of 12.5 and 20% respectively. The renin assay kit (reference range: 0.2-2.8 ng/mL/hr supine; 1.5-5.7 ng/mL/hr upright) was characterised by an intra-assay CV of 4% and an inter-assay CV of 7.3%. Sensitivity was deemed at <20 pg/mL (95% confidence limit). Blood samples for NT-pro-BNP and aldosterone were collected in vacuum collection tubes containing lithium-heparin. They were immediately kept on ice and spun for 10 minutes at 3000 rpm at 4ºC. Two 1mL plasma aliquots (one for each of NT-pro-BNP and aldosterone) were then collected in a 1.5 mL plastic tubes, and frozen at -70ºC, pending formal analysis. The aldosterone assay kit (reference range: 75-150 pg/mL supine; 35-300 pg/mL upright) gives an intra-assay coefficient of variation (CV) of 5.5% and an inter-assay CV of 5.2%. Sensitivity is deemed at <20 pg/mL (95% confidence limit). The assay for NT-pro-BNP is characterised by an intra-assay CV of 10%. All blood samples for biomarkers were measured in the supine position following an hour's rest. Individual biomarker assay methodology is outlined in table 2.2.

Table 2.2 - Biomarker assay methodology

Assay	***System***	***Method principle***
VEGF	R and D systems	ELISA
ANP	Bachem	Radioimmunoassay
BNP	Bachem	Radioimmunoassay
NT-pro-BNP	Oxford Biosystems	ELISA
Aldosterone	Diasorin	Radioimmunoassay
Renin	Diasorin	Radioimmunoassay
High sensitivity copeptin	B.R.A.H.M.S. Kryptor	Time-Resolved Amplified Cryptate Emission (TRACE)

2.12.4 Echocardiography (including tissue doppler)

At the start of this study visit, the patient underwent echocardiography enabling a baseline assessment of left ventricular function in moderate low sodium states. This procedure was repeated after infusion of one litre of 0.9% saline for each participating subject, allowing additional assessment of cardiac function in response to acute salt loading. All echocardiographic measurements were carried out by Dr. Adnan Nadir (Clinical Research Fellow, Division of Cardiovascular and Diabetes Medicine, Ninewells Medical School).

The Philips iE33 echocardiography system enables semi-automated analysis of true left ventricular volumes, using all the voxels to generate a full three dimensional endocardial border. This approach is characterised by higher accuracy and less dependency on left ventricular shape assumptions than conventional methods. Its three dimensional quantification advanced (3D QA) waveform display provides accurate data for the assessment of global function based on left ventricular volume, ejection fraction and stroke volume, while allowing simultaneous display of 17 regional waveforms, enabling temporal comparisons between the segments. Any patients found to have previously-undetected baseline left ventricular systolic ejection fraction below 40% were excluded by protocol at this stage of this study. The E-wave/A-wave (E/A) ratio was used to assess left ventricular systolic and diastolic function. E prime (E') was measured at the level of the mitral valve annulus as a sensitive index of longitudinal axis left ventricular relaxation [503]. The latter method is well established in the University of Dundee Division of Medicine and Therapeutics. Three dimensional echocardiography also allowed accurate,

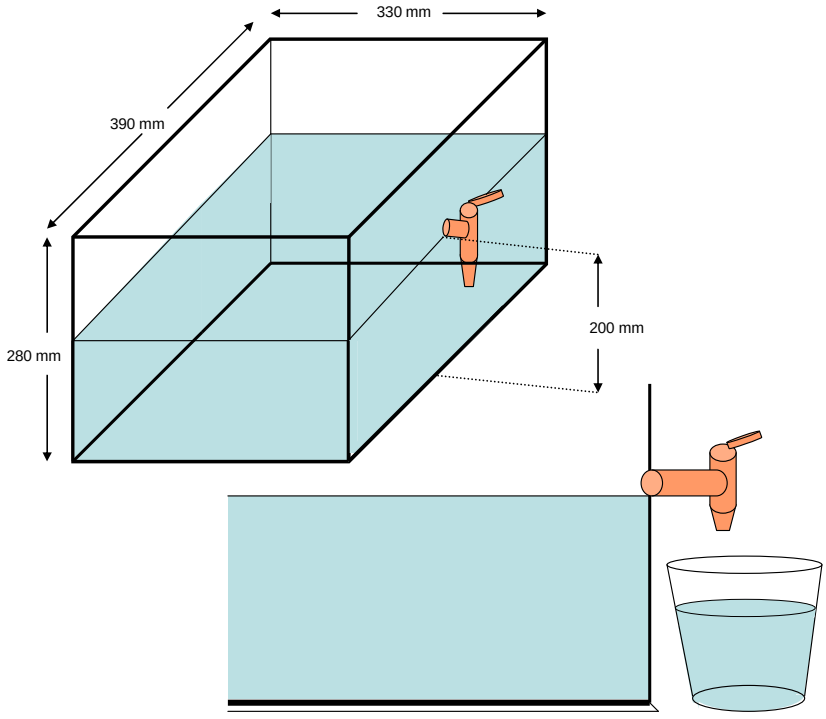
assumption free and reproducible quantification of left ventricular mass. The 3D QA waveform display was thus poised to characterise the baseline echocardiocardiographic features of thiazolidinedione 'tolerant' and 'intolerant' patients, and to investigate the effects of dietary and therapeutic interventions on cardiac function among patients in either cohort.

2.12.5 Ankle-foot volume measurements

AFV was measured close to the start of visit 2, and repeated after infusion of one litre of 0.9% saline, allowing analysis of data in both low and acute high sodium states. Measurements were made using a plastic water bath (measuring 390 mm long by 330 mm wide by 280 mm high) with an outlet tap for water overflow at the top of the bath, located 200 mm from the bottom of the water bath (figure 2.1). This tap had a tube attached, from which the overflow water was collected into a plastic container. Patients were verbally familiarised with the procedure prior to commencing the measurements. The water bath (including all its grooves) was filled with water at 26-27 °C, and water was allowed to flow out through the overflow tap into the plastic collecting container until the water within the water bath levelled with the overflow tap. The latter was fully closed at this point. Water temperature was assessed using an electronic thermometer. The plastic collecting container was then emptied and weighed on an electronic scale, ensuring it was placed at the centre of the weighing scale platform, without touching the bath or its attached water tap. The subject was then asked to dip their bare feet slowly into the bath of water until their feet were flat at the bottom of the bath, as they sat at the edge of a bed with their knees flexed at right angles. His/her feet were positioned into a reproducible

position, facing forwards, in the water bath. The subject was then asked to sit still, while being kept comfortable to rest their arms on a pillow placed over their knees. Once the water level within the bath had settled, the water tap was turned open and left in this position for five minutes (timed using an electronic stopwatch). The volume/weight of the water displaced was weighed at the end of this time-interval. The procedure was repeated thrice, enabling the calculation of mean values for the ankle fluid volume at each stage of the study. Displaced water was replaced within the water bath at each repeat ankle fluid volume measurement procedure.

Figure 2.1 – Schematic diagram of a water bath used to measure ankle-foot volume by water displacement



2.12.6 Pulse wave velocity and analysis

Arterial stiffness was measured in this study using applanation tonometry (SphygmoCor®). The latter is a computerised diagnostic tool permitting accurate description of pulse wave characteristics and pulse wave velocity, and the extrapolation of findings to central cardiac and aortic physiological events. SphygmoCor® derives central aortic pressure waveform non-invasively from the pressure pulse recorded at a peripheral site by applanation tonometry. The apparatus reconstructs the aortic waveform from the non-invasively derived radial waveform by a validated mathematical model termed transfer function [504]. While the characteristics of transfer function are determined by the physical properties of the arterial system (namely arterial diameter, wall elasticity, wall thickness, amount of branching and the condition of the peripheral arterial beds), its main components do not change markedly between normal individuals with age. This is consistent with the observation that most of the ageing changes occur in the aortic trunk rather than in the arteries of the arm [505].

Although arterial stiffness is a major risk factor for cardiovascular disease, and predicts the development of left ventricular failure [506-508], traditional methods detecting left ventricular failure do not provide information on the arterial dynamics that determine left ventricular hypertrophy. To this effect, this study utilized the technique of applanation tonometery to investigate the hypothesis that individuals prone to develop HF after incident thiazolidinedione prescription are characterised by greater arterial stiffness compared with their ‘thiazolidinedione tolerant’ counterparts.

The patient was advised to lie supine, calm and relaxed on a bed, with their head supported on a pillow and their arms relaxed by their sides, in a temperature controlled room. The patient's right wrist was supported, such that the palm faced upwards. I ascertained that the radial pulse was identical in both arms and that the arterial pressure by cuff sphygmomanometry was within 10mmHg systolic. A baseline ECG ruled out significant arrhythmias while baseline echocardiography carried out immediately prior to this procedure rule out significant aortic stenosis (gradient > 60 mmHg). Both aortic stenosis and cardiac arrhythmias adversely affect the reproducibility of pulse wave analysis and velocity measurements [509, 510]. For pulse wave analysis, the SphygmoCor® tonometer was placed on the patient's radial artery by the clinical research fellow. The patient was advised to dorsiflex the wrist while supporting it on a small cushion, so as to push the artery towards the surface, easing access. The tonometer was pressed gently and steadily on the patient's radial artery, adjusting the tonometer slightly backwards and forwards until a consistent large arterial waveform was displaced completely within the laptop computer monitor screen. The pulse wave signal was captured only after ascertaining that the pulse waveform was characterised by a steady vertical waveform position, constant pulse height and consistent waveform profiles for two complete screens (at least 10 seconds). The study report was generated by the computer software. Data were recorded on the CRF.

For pulse wave velocity measurement, which was carried out immediately following pulse wave analysis, the patient was positioned as previously. Three ECG electrodes were attached to the patient's skin. Skin was prepared beforehand, by shaving excess

hair over the electrode site (if indicated) and briskly rubbing the site with a cotton pad soaked in isopropyl alcohol, to ensure a stable, artefact free ECG trace. The SphygmoCor® system uses a LEAD II ECG lead configuration system. Leads were placed on the chest wall to increase QRS height. The pulse wave velocity was calculated using a three-stage process. The distance from the suprasternal notch to the arterial pulse site was measured and recorded in millimetres in the SphygomoCor® computer software. The distance between the suprasternal notch and the carotid pulse was likewise measured and recorded. The subtraction of these two measurements was automatically performed by the software once the proximal and distal values were entered. The tonometer was used to capture steady pulse waveforms, intially on the distal (radial artery) site, and subsequently on the proximal (carotid artery) site, once good quality waveforms were ascertained for each site. The study report was then generated by the computer software. Data were recorded on the CRF.

2.12.7 Assessment of glomerular filtration rate (inulin clearance method)

Glomerular filtration rate (GFR) cannot be measured directly in humans, and is determined by measuring the clearance of an ideal filtration marker. Inulin, an uncharged polymer of fructose derived from plant tubers, fulfils this requirement on account of the following characteristics, which render it the gold standard method in this field [511, 512]:

(i) its low molecular weight

(ii) physiologically inert

(iii) being unbound to plasma proteins

(iv) ability to reach a stable plasma concentration

(v) free filtration at the glomerulus

(vi) not reabsorbed, secreted or metabolised in the kidney

(vii) does not alter renal function

The CV in serum and urine inulin levels ranging from 100 to 250 mg/L is less than 5%. The intra-test CV in inulin clearance is around 10% [512].

As outlined earlier, following informed consent, patient participants were requested to fast from 22.00 hours prior to visit 2, refraining from smoking and consuming alcohol and caffeine containing beverages for the duration of the fast (free fluids permitted). On arrival for visit 2, two intravenous cannulae were inserted into the antecubital veins, one for infusion of inulin, and the second one into the contralateral vein for drawing blood. The patient was made comfortable on a bed, and was advised to remain supine throughout the test procedure, except for voiding. Baseline levels of inulin were measured at t = -130 minutes. Blood samples were collected in a Z serum clot activator vacuum collection tube with gel separator and allowed to settle at room temperature for about ten minutes. They were then spun for ten minutes at 3000 rpm and stored at -20ºC, prior to transfer on dry ice for analysis by Mr Neil R Johnston in Professor David Webb's laboratory at the Clinical Pharmacology Unit, Queens' Medical Research Institute, University of Edinburgh, Edinburgh. Plasma inulin was measured using an in-house colorimetric microplate assay based upon the chemical reaction between fructose and resocinol, following an initial acid hydrolysis of inulin to its fructose subunits [513]. This method gives sensitivity of 50 ug/mL, an intra-assay CV of 3.7% and an inter-assay CV of 5.35%. A priming dose of inulin (Inutest® 25%) was commenced at t = -120 minutes,

administered as an intravenous bolus of 50 mg/kg inulin, followed by a continuous intravenous infusion at a rate of 25mg/min, infused in 0.9% saline until t = 130 minutes (ie over 250 minutes) [514]. Venous blood samples for plasma inulin were again measured at t = - 10 minutes and t = - 5 minutes. Plasma levels of inulin reach a steady state after approximately 60 to 90 minutes of administration [512, 514]. One litre of 0.9% saline was then infused over two hours (as per salt loading protocol, section 2.12.11), commencing at t = 0 minutes, with measurements of plasma inulin levels at t = 120 minutes and t = 130 minutes. The patient's glomerular filration rate was estimated from the steady state infusion of inulin according to the calculation method described by Schnurr et al. [515].

Normally, clearance (C) is calculated from serum and urine samples using the formula:

U*V/ S ml/min

where U = urine concentration, V = urine volume and S = serum concentration

In the method outlined here, clearance is calculated by replacing U*V by the infusion rate **IC*IV**

where IC = concentration of the test substance in the infusion fluid and IV = rate of the infusion.

i.e. C = IC*IV/ S ml/min

The result was corrected for body surface area using the standard nomogram.

Inulin is not considered a hazardous compound according to EU Directive 67/548/EC. Therefore, any risks associated with inulin infusion were related only to the procedures of intravenous cannulation and infusion. Inulin had been infused in the same vascular research laboratory on several occasions, without adverse effects [516].

2.12.8 Fractional excretion of sodium

Fractional excretion of sodium (FENa) was calculated from spot measurements of urine sodium, serum sodium, urine creatinine and plasma creatinine, using the formula:

$$\mathbf{FENa = (U_{Na}*P_{cr}/P_{Na}*U_{cr})*100}$$

where U_{Na} = urine sodium, P_{cr} = serum creatinine, P_{Na} = serum creatinine and U_{cr} = urine creatinine [517].

All four measurements were made at t = -120 minutes and t = 0 minutes (before infusion of 0.9% saline), enabling a calculation of mean FENa at low sodium states). Similar measurements were made at t = 120 minutes and t = 240 minutes (after 0.9% saline infusion, enabling a calculation of mean FENa following acute high salt loading.

2.12.9 Fractional excretion of lithium

Renal reabsorption of lithium is virtually confined to the proximal tubules, and occurs in the same proportion as that of sodium and water. Post-proximal tubule reabsorption of lithium has been deemed limited [518, 519]), and probably unimportant in humans [520]. This method has been deemed the best available estimate of proximal tubule function [521]. Hence calculation of fractional excretion of lithium (FELi) gives an accurate and non-invasive assessment of sodium and water delivery to the distal tubules.

FELi was likewise calculated from spot measurements of urine lithium, serum lithium, urine creatinine and plasma creatinine , using the formula

$$\mathbf{FELi = (U_{Li}*P_{cr}/P_{Li}*U_{cr})*100}$$

where U_{Li} = urine lithium, P_{cr} = serum creatinine, P_{Li} = serum lithium and U_{cr} = urine creatinine

Once again, all four measurements were made at t = -120 minutes, t = 0 minutes, t = 120 minutes and t = 240 minutes, enabling calculation of FELi at low sodium and acute high sodium states.

2.12.10 Fractional reabsorption of distally delivered sodium

Fractional reabsorption of distally delivered sodium (FRDDNa) was calculated at t = -120 minutes, t = 0 minutes, t = 120 minutes and t = 240 minutes, using the formula:

(FELi - FENa/FELi)*100

Each result was expressed as a percentage [522, 523].

2.12.11 Salt sensitivity of blood pressure

Pulse, blood pressure and respiratory rate readings were taken at ten minute intervals from the non-dominant arm using an automated sphygmomanometer while a litre of 0.9% saline was infused over two hours in the recombinant position, as discussed earlier. The attending clinical research fellow also assessed the patient for signs of fluid overload at each time-point. Participants complaining of dyspnoea or whose respiratory rate exceeded 20 breaths per minute at rest (or increased by more than five breaths per minute from baseline) were assessed earlier. Patients with *a priori* HF were excluded from the study. Moreover, baseline echocardiography performed at the start of visit 2 excluded patients whose left ventricular ejection fraction was estimated at less than 40%. The saline infusion was discontinued immediately if patients were deemed to be developing signs of fluid overload, showing other signs of decompensation, or developing a blood pressure rise exceeding 170/95 mmHg at rest (mean of two duplicates).

2.13 Study procedure - visit 3

Visit 3 was scheduled one to two weeks after visit 2, following five days on a 'high normal' 200 mmol/day sodium diet, essentially comprising the previous 'low salt' diet supplemented by ten slow sodium tablets (HK Pharma, each 10 mmol/sodium) daily. On these five preceding days, participating subjects were asked to collect an early morning urine sample for urinary sodium and creatinine estimation. They were also instructed to weigh themselves on awakening, dressed in their underwear, using the electronic self-weighing scale provided, recording the measurements on a diary. Patients arrived at the vascular research laboratory at around 08.30 hours, having consumed 300 mg lithium carbonate at 22.00 hrs and subsequently fasted the previous night. Patients were asked to refrain from smoking and consuming alcohol and caffeine-containing beverages for the duration of the fast; compliance to this advice was verbally ascertained at the start of the study visit. On arrival, patients were made comfortable on a bed, and remained supine, except for voiding and ankle-fluid measurements. Patients remained fasted until the end of the study visit, which lasted until about 13.30 hours. They were provided with a sandwich meal before leaving the research unit.

During this study visit, the following baseline assessments and measurements were repeated:

- Echocardiography including tissue Doppler
- Concomitant pharmacological therapy
- Compliance with a 'high normal' 200 mmol/day sodium diet
- Compliance with medication

- Assessment of occurrences of hypoglycaemia
- Dipstick urinalysis and urine for microalbuminuria
- Weight/waist measurement
- Blood pressure (in triplicate)
- AFV by water displacement
- Pulse wave analysis and velocity.
- HbA1c
- Plasma for biochemistry (urea and electrolytes, liver function tests)
- ANP
- Aldosterone
- Renin
- BNP and NT-proBNP
- VEGF
- AVP

2.13.1 Total body water estimation

Total body water (TBW), comprising both intracellular and extracellular fluid, was measured in this study visit using deuterium, a natural stable isotope of hydrogen. The isotope dilution technique has been dubbed as the most robust method of TBW estimation [524-526], with a reproducibility of approximately 0.5% [525]. A basal spot urine sample was collected at the onset of study visit 3. A 25 ml aliquot of this sample was stored in a labelled universal bottle in a freezer for eventual analysis by Ms. Alexandra Small in Professor Tom Preston's Stable Isotope Biochemistry Laboratory, Scottish Universities Environmental Research Centre (SUERC),

Glasgow. 4g deuterium oxide were administered as an oral stable isotope dilution at t = -120 minutes, ie 2 hours prior to the administration of the inulin infusion. Deuterium oxide had been previously produced gravimetrically at SUERC, diluted with around 50 ml tap water and stored in a leak proof container in a specific freezer until thawed for use. The dose bottle was rinsed with tap water, and the latter was also drunk by the patient, ensuring complete ingestion of the deuterium oxide dose. The participating patient was asked to provide three post dose urine samples at approximately two hourly intervals (approximately t = 0 minutes, t = 120 minutes and t = 240 mins). from the start of the inulin infusion. Patients were encouraged to void at an earlier stage or at additional time points if they so required. The time and volume of each sample was accurately recorded and a ~25mL aliquot stored in a labelled universal bottle in a freezer for IRMS analysis at SUERC. The residue of each sample was discarded. Patients were permitted to drink a volume of water equivalent to urinary losses throughout this visit.

Fat-free mass (FFM, also known as lean body mass) was derived from TBW by dividing the latter by the water content of fat free tissue (73.2%) [524]. Fat mass (FM) was derived by subtracting FFM from each individual patient's total body mass. Percentage FM and percentage FFM were calculated relative to total body weight [527]. Derived FFM values were validated against non-linear regression models published by Wang et al. (FFM = 10.8*height (m) $^{2.95}$ for males and 10.1*height (m) $^{2.90}$ for females) [528].

Use of deuterium for TBW measurement has been deemed free from the hazards associated with radioisotopes. An adult male of 80kg may have a TBW of 40 kg or

greater. This will naturally contain 155 ppm deuterium or 6.2g deuterium oxide in 40 kg water [527]. Isotope ratio mass spectrometry employed for TBW estimation allowed minimalization of deuterium dosage, such that all doses used were less than that naturally present in the human body. This approach, coupled with the sourcing of deuterium oxide of guaranteed purity and the use of a non-invasive protocol combining oral doses of heavy water and urine sampling, ensured that the TBW protocol was completely risk free.

2.13.2 Glomerular filtration rate

Glomerular filtration rate was once again assessed in visit 3 using the inulin clearance method outlined earlier. Following a bolus dose (50 mg/kg), inulin was administered at a rate of 25 mg/min over 130 minutes (starting at t = 0 minutes). Venous plasma samples were withdrawn pre-infusion (t = -10 minutes), t = 120 minutes and t = 130 minutes, and sent for measurement of inulin levels.

2.13.3 Salt and water handling techniques

FENa, FELi and FRDDNa were calculated on a chronic moderately high sodium diet using the formulas discussed earlier. The relevant urine and serum samples were collected at t = -120 minutes, t = 0 minutes, t = 120 minutes and t = 130 minutes.

2.14 Biostatistical considerations

2.14.1 Sample size

About five months into recruitment for this study, Wyeth Pharmaceuticals, who had provided funding via the Scottish Translational Medicines Research Collaboration (TMRC) underwent a merger with Pfizer. An initial threat that funding would be completely withdrawn was successfully challenged on ethical grounds (given that patients had already been enrolled and undergone invasive procedures). However, the project had to be scaled down to a to a maximum of 30-40 patients, enabling a comparison between 10 thiazolidinedione intolerant patients and 20 thiazolidinedione tolerant completed patients, which was a significant reduction from the original recruitment plan for this study (40-60 patients comprising at least 20 thiazolidinedione tolerant and 20 thiazolidinedione intolerant patients). It should also be acknowledged that recruitment had proved more difficult than anticipated, especially for thiazolidinedione intolerant patients (as confirmed section II), despite adopting an integrated and multifaceted approach.

This was an exploratory study aimed at assessing the potential of ankle−foot volume (and/or other specified measurements) under acute and/or chronic sodium challenge as biomarkers of TZD intolerance. This was of interest to Wyeth Pharmaceuticals, who provided funding via the TMRC. We did not find data in the literature to permit a formal power calculation for ankle-foot volume measurements. The background literature from which the sample size was derived was a study in which AFV had a between-day intra-subject coefficient of variation of 1.76% [529];

amlodipine therapy increased this parameter by 23% in 80 unselected hypertensive patients in a study by Fogari et al [530]. I found no previous data measuring AFV on high salt diets either in TZD-tolerant or intolerant patients with or without diabetes. Other endpoints were purely exploratory. It was pre-specified that there would be no adjustment for multiple comparisons.

Once the study had been initiated, as already mentioned (page 142), following the merger of Wyeth and Pfizer, subsequent limitations on funding imposed by the TMRC dictated a reduced sample size from that originally intended. Thus, a revised submission was made to the TMRC adopting a more constrained exploratory analysis defining a reference range (with 95% CIs) from thiazolidinedione tolerant patients, and plotting individual data from the thiazolidinedione intolerant patients individually against these reference ranges to examine and explore the data formally for trends [531]. The ultimate power of these analyses was lower than originally intended and can be visualised for those results that were positive from the graphs showing 95% CIs for the reference range in the TZD-tolerant patients.

2.14.2 Statistical analyses

Objective 1: All patients who completed visit 1 were considered in the analysis of baseline characteristics. Any patient(s) withdrawing consent after visit 1 had their data included in the analysis.

Objective 2: All patients who completed all the study visits, or had completed visits 1 and 2 (including patients who have withdrawn due to hypertension) were

considered in the analysis. If the patient withdrew consent after visit 2, their data were included in the analysis. Patients who withdrew prior to visit 2 were not considered in the analysis.

Patients judged not to have complied with diet on the basis of urinary sodium excretion were excluded from the analysis. Given the limited sample size, only descriptive statistics were used.

2.15 Follow-up of these patients

All cohort 1 patients were offered the option of switching back from gliclazide to their usual thiazolidinedione at completion of visit 3. They were also advised to recommence their usual antihypertensive therapy, and switch back to their 'usual' sodium diet. All patients were offered a follow-up visit within a few days of completing visit 3, so as to address issues pertaining to drug therapy modification, glucose monitoring or any other potential queries arising out of their participation in this study. General practitioners were advised regarding any long-term therapeutic modifications once patients completed their participation in this study.

2.16 Validation of ankle-foot volume measurement by the water displacement technique

2.16.1 Aim

The water displacement technique is establishing itself as a useful volumetric method for monitoring peripheral oedema. However, it is also considered to be time consuming and difficult to perform [532, 533]. Its day-to-day reproducibility has only been validated over a mean duration of 4.8 days [529], which limits its use to monitor longer-term volumetric changes. This is particularly relevant in research practice, where rigorous objective assessment is crucial. This study aimed to investigate intra-subject variability of the water displacement technique over a longer period of 2 weeks.

2.16.2 Methods

Ten healthy individuals without signs of peripheral oedema were recruited for this single centre prospective cohort study carried out at the Vascular Research Laboratory of the Department of Clinical Pharmacology and Therapeutics, Division of Cardiovascular and Diabetes Medicine, Medical Research Institute, University of Dundee. Recruitment was carried out by emailing potentially interested participants working at Ninewells Medical School. No particular instructions on physical activity, working hours or break time was given to participants. Subjects were excluded if any of the following criteria were met: current hospitalization, known history of selected medical conditions (hypertension, cardiac failure, renal

impairment/failure, liver disease, lymphoedema, chronic venous insufficiency, deep vein thrombosis), treatment with diuretics, calcium channel antagonists, statins, insulin or thiazolidinediones, known pregnancy, presence of superficial skin ulcers, open sores, wounds, or other skin conditions on the lower extremity, history of an ankle injury or lower extremity surgery within the past 30 days.

I carried out a simplified clinical examination of the lower limbs, essentially comprising an assessment for signs of chronic venous insufficiency, ulcers, ankle/leg injuries or skin conditions that precluded subjects from participation.

Subjects fitting inclusion criteria had their cumulative (bilateral) ankle volume measured in triplicate at weekly intervals for three successive weeks. Measurements were taken at approximately the same time each week (± 1 hour), minimising diurnal variation. An outline of the method used has been described elsewhere. All measurements were carried out by myself. Shoes and socks were removed before each examination. Height, weight and blood pressure were measured for each participant. Standing height was measured using a stadiometer and standing weight using the same validated electronic scale, as outlined earlier. Blood pressure was measured using a British Hypertensive Society validated automated sphygmomanometer with the patient sitting comfortably at rest for five minutes. Waist circumference was measured using a non-elastic measuring tape in accordance with Scottish Diabetes Research Network (SDRN) standard operation procedures.

Distribution of baseline demographic data and clinical characteristics were presented as mean (± SD) or as percentages. An estimate of the analytical variance (also

known as measurement error), defined as the average variance of repeated measurements at the same time point, was defined for the cohort of participating subjects at each study visit using one way ANOVA (subject as term). Within-individual variance, the average variance of repeated measurement in the same subject at different time points was likewise calculated for each individual using two-way ANOVA (subject and day as terms). Residuals were deemed to be normally distributed using the Kolmogorov-Smirnov test and by constructing Q-Q plots. The CV in each case was calculated by dividing the square root of the total error term of the adjusted mean squares from ANOVA by the mean of the observations and expressed as a percentage. Intra-class correlation coefficient (ICC) (with corresponding 95% CI value) was calculated as an overall estimation of the reproducibility of leg volume measurements. All statistical analyses were performed using SPSS® version 21.0.

2.16.3 Results

Descriptive statistics for the ten participating subjects (five males, five females) are summarised in table 2.3. Although mean (SD) BMI was in overweight range, mean systolic blood pressure (SBP) and diastolic blood pressure (DBP) readings were in the normotensive range.

Table 2.3 – Demographic and clinical characteristics of the ankle-foot volume validation study participants (n = 10)

Subject characteristic[a]	***Mean (SD)[b] or absolute value[c]***
Age (years)	41.80 (9.3)[b]
Females	5 (50%)[c]
Weight (kg)	77.4 (13.2)[b]
Body mass index (kg/m^2)	26.5 (3.9)[b]
Waist circumference (cm)	76.2 (21.6)[b]
Resting heart rate (min^{-1})	68.5 (10.2)[b]
Resting systolic blood pressure (mmHg)	123.2 (11.9)[b]
Resting diastolic blood pressure (mmHg)	75.6 (6.8)[b]

[a]*Data accrued from all participants in each of the three study visits.*

Tables 2.4, 2.5 and 2.6 summarise individual and mean (± SD) AFV measurements for each of the ten participating subjects at each of the three study visits. CV for individual subjects ranged from 0.62% to 3.73% in visit 1, 0.70% to 2.72% in visit 2 and 0.66% to 2.59% in visit 3. Cumulative CV for all participating subjects was 1.96% , 1.66% and 1.57% for visits 1, 2 and 3 respectively. The corresponding ICC values were 0.995 (95% CI 0.986, 0.999), 0.997 (95% CI 0.992, 0.999) and 0.997 (95% CI 0.992, 0.999). Mean (± SD) AFV measurements for individual patients over the cumulative observation period of two weeks are plotted on figure 2.2. Plots of the difference in AFV measurements between two individual study visits against the mean AFV for these study visits are given in figures 2.3 to 2.5, as a visual appreciation of the amounts of variability which can be expected using this technique.

Table 2.4 - Leg volume measurements and derived coefficient of variation for each subject at visit 1

Subject number	AFV_{1a}	AFV_{1b}	AFV_{1c}	$AFV_{mean\ 1}$	CV_1
1	2761	2904	2911	2858.7 (84.7)	2.96
2	2990	2998	2944	2977.3 (29.1)	0.98
3	3617	3755	3702	3691.3 (69.6)	1.89
4	3524	3557	3566	3549.0 (22.1)	0.62
5	3275	3252	3211	3246.0 (32.4)	1.00
6	2396	2412	2281	2363.0 (71.5)	3.02
7	2921	2735	2746	2800.7 (104.4)	3.73
8	2514	2410	2473	2465.7 (52.4)	2.12
9	3648	3673	3609	3643.3 (32.3)	0.89
10	3353	3386	3367	3368.7 (16.6)	0.49

AFV, ankle-foot volume (mls); AFV_{1a}, first ankle-foot volume reading for visit 1; AFV_{1b}, second ankle-foot volume reading for visit 1; AFV_{1c}, third ankle-foot volume reading for visit 1; $AFV_{mean\ 1}$, mean (SD) ankle-foot volume for visit 1; CV_1, coefficient of variability for visit 1(%)

Table 2.5 - Leg volume measurements and derived coefficient of variation for each subject at visit 2

Subject number	AFV_{2a}	AFV_{2b}	AFV_{2c}	$AFV_{mean\ 2}$	CV_2
1	2777	2742	2774	2764.33 (19.40)	0.70
2	2783	2924	2912	2873.00 (78.17)	2.72
3	3767	3617	3613	3665.67 (87.78)	2.39
4	3615	3664	3675	3651.33 (31.94)	0.87
5	3342	3433	3335	3370.00 (54.67)	1.62
6	2306	2347	2376	2343.00 (35.17)	1.50
7	2753	2768	2786	2769.00 (16.52)	0.60
8	2367	2411	2372	2383.33 (24.09)	1.01
9	3774	3711	3729	3738.00 (32.45)	0.87
10	3357	3474	3435	3422.00 (59.57)	1.74

AFV, ankle-foot volume (mls); AFV_{2a}, first ankle-foot volume reading for visit 2; AFV_{2b}, second ankle-foot volume reading for visit 2; AFV_{2c}, third ankle-foot volume reading for visit 2; $AFV_{mean\ 2}$, mean (SD) ankle-foot volume for visit 2; CV_2, coefficient of variability for visit 2(%)

Table 2.6 - Leg volume measurements and derived coefficient of variation for each subject at visit 3

Subject number	AFV_{3a}	AFV_{3b}	AFV_{3c}	$AFV_{mean\ 3}$	CV_3
1	2734	2743	2797	2758.00 (34.07)	1.24
2	3074	2988	3040	3034.00 (43.31)	1.43
3	3520	3534	3489	3514.33 (23.03)	0.66
4	3738	3654	3733	3708.33 (47.12)	1.27
5	3355	3267	3321	3314.33 (44.38)	1.34
6	2312	2234	2301	2282.33 (42.22)	1.85
7	2710	2803	2852	2788.33 (72.13)	2.59
8	2315	2359	2311	2328.33 (26.63)	1.14
9	3658	3716	3644	3672.67 (38.18)	1.04
10	3521	3394	3395	3436.67 (73.04)	2.13

AFV, ankle-foot volume (mls); AFV_{3a}, first ankle-foot volume reading for visit 3; AFV_{3b}, second ankle-foot volume reading for visit 3; AFV_{3c}, third ankle-foot volume reading for visit 3; $AFV_{mean\ 3}$, mean (SD) ankle-foot volume for visit 3; CV_3, coefficient of variability for visit 3(%)

The overall CV of the AFV measurement technique observed over a period of 2 weeks (spanning from visit 1 to visit 3) stood at 1.74%. The corresponding ICC value was 0.995 (95% CI = 0.985, 0.999).

Figure 2.2 – Mean (SD) ankle-foot volume values in ten healthy subjects measured at each of three successive visits (1-3) one week apart

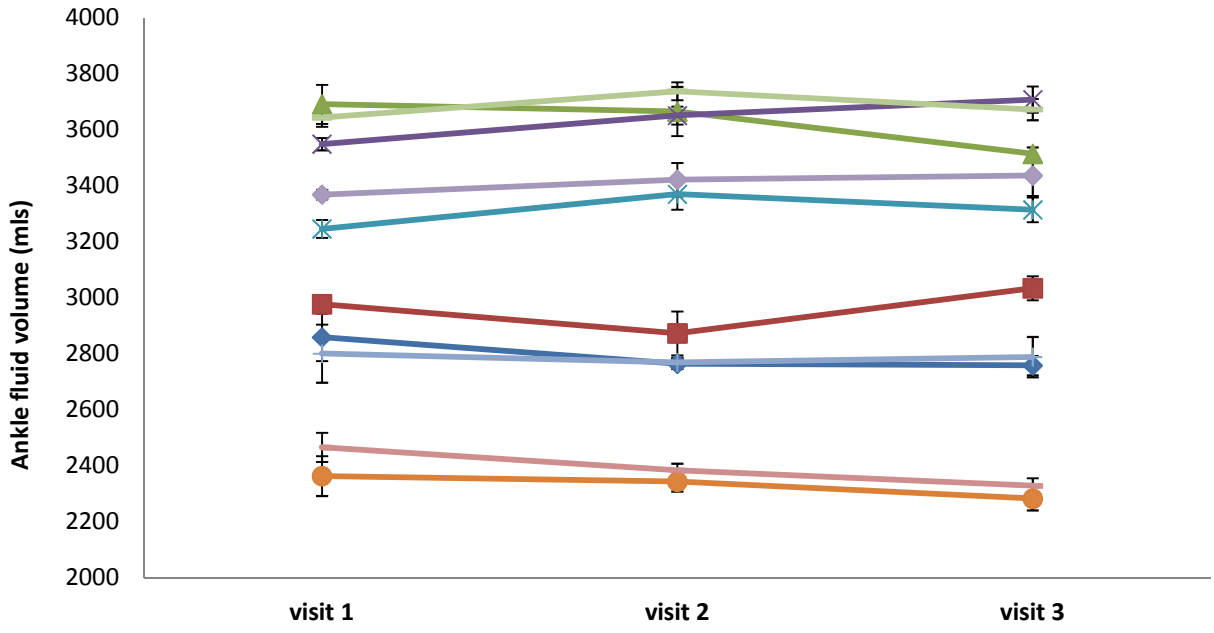
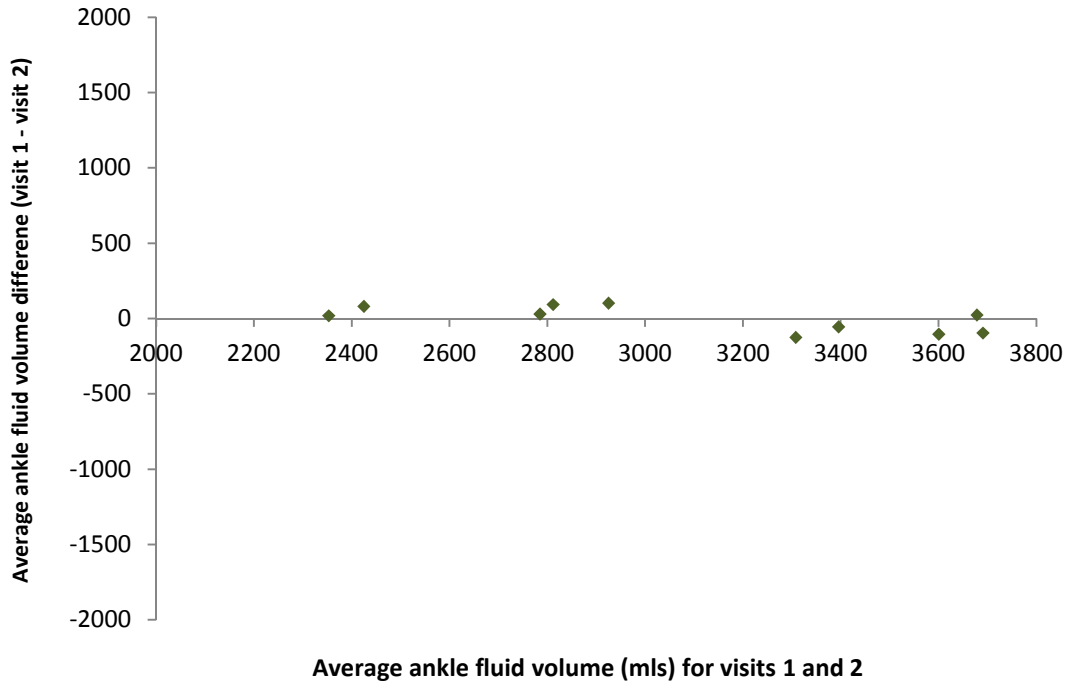
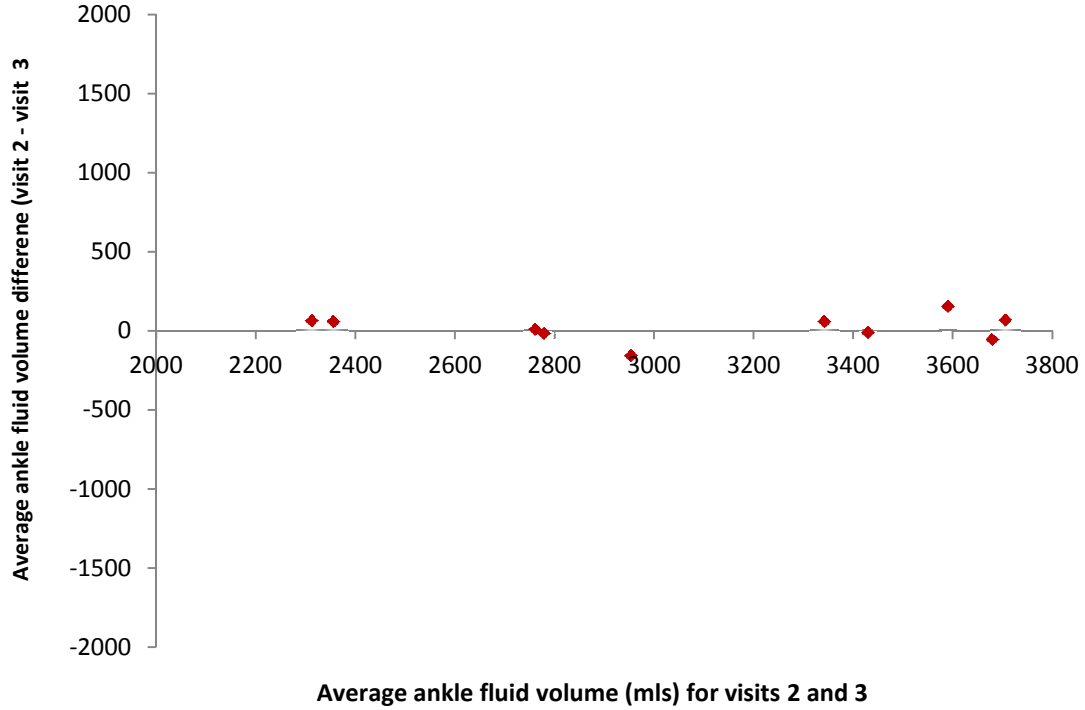


Figure 2.3 – Variation in ankle-foot volume measurements between visits 1 and 2 [CV = 0.91% for visits 1 and 2[a]; ICC = 0.993 (95% CI 0.974, 0.998)]



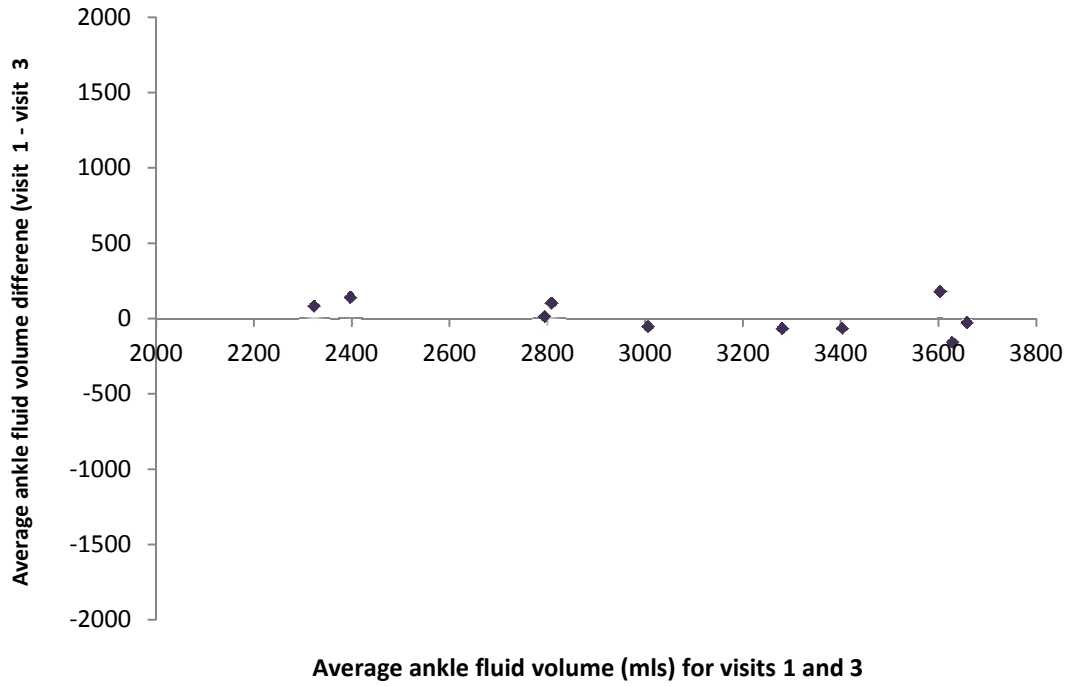
[a]*one-way ANOVA (subject and day as terms)*

Figure 2.4 – Variation in ankle-foot volume measurements between visits 2 and 3 [CV = 0.81% for visits 2 and 3[a]; ICC = 0.994 (95% CI 0.977, 0.999)]



[a]*one-way ANOVA (subject and day as terms)*

Figure 2.5 – Variation in ankle-foot volume measurements between visits 1 and 3 [CV = 0.89 % for visits 1 and 3[a]; ICC = 0.989 (95% CI 0.958, 0.997)]



[a]*one-way ANOVA (subject and day as terms)*

2.16.4 Discussion

This study demonstrated that the water displacement technique is a relatively easy, yet reproducible method of measuring ankle-fluid volumes in human subjects. Cumulative CV for all participating subjects ranged from 1.96% in visit 1 to 1.57% in visit 3, which compares well with those reported in the literature. In a study on patients with lymphoedema, Auvert and Vayssairat reported a reproducibility of 1.3% for the water displacement technique [534]. Van Hamersvelt and colleagues report use of a water displacement device with a lower CV value of 0.30% [535]. This study reports ICC values of 0.995 (95% CI 0.986, 0.999), 0.997 (95% CI 0.992, 0.999) and 0.997 (95% CI 0.992, 0.999) for visits 1, 2 and 3 respectively. These values compare excellently with those reported by Brodovicz et al. (0.93-0.96) in a

study which evaluated foot and ankle volumes in each leg separately [536]. Additionally, this study's CV and ICC values confirm that measurements are highly reproducible over a period of two weeks in the absence of any significant intervention. This renders interpretation of temporal effects on AFV using this measurement technique highly plausible in an experimental setting. To this effect, Brijker et al. reported a CV of 1.76% over a mean observation period of 4.8 days [529], which is considerably shorter than this study's observation period of two weeks. Diurnal variation in leg volume, and hence fluid displacement have been reported in several studies [529, 537, 538]. This possibility was minimised by ensuring that participating subjects had their fluid volumes measured at approximately identical times each week [539]. None of the subjects reported significant alteration in their daily lifestyle over the intervening observation period.

2.16.5 Conclusion

Measuring leg volume by water displacement is relatively easy, cheap, and highly reproducible. It can be used to monitor temporal changes in peripheral oedema over an extended period of time.

Section II - Results

2.17 Phenotype

2.17.1 Baseline demographic characteristics

Thirteen Caucasian patients attended and completed the initial screening visit for this study (visit 1). Of these, 11 (subjects 1 to 9, subject A and subject B, comprising seven males and four females) reported themselves to be tolerant of thiazolidinediones, and two (subjects 11 and 12, both females) had been withdraawn from TZDs on account of fluid retention. In one of the latter cases, thiazolidinedione had been withdrawn less than three months after index thiazolidinedione prescription on account of 'severe bilateral hand oedema'. The second had discontinued her thiazolidinedione within one to two weeks after developing 'weight gain and severe abdominal, bilateral upper limb and ankle swelling'. Adverse effects resolved spontaneously on drug withdrawal in both instances. Thiazolidinedione tolerant subject A had to be withdrawn soon after recruitment into this study on account of development of proteinuria soon after withdrawal of his losartan therapy (as per study Protocol). Subject B was likewise withdrawn after developing an excessively high blood pressure (> 160/110 mmHg) on withdrawal of his antihypertensive (atenolol 50 mg). Thiazolidinedione tolerant subjects were on average older [mean (95% CI) age = 61.6 (58.9, 64.2) years] than their thiazolidinedione intolerant counterparts (both aged 55) at recruitment into the study (table 2.7). Thiazolidinedione 'intolerant' subject 10 had been diagnosed slightly earlier (166 months) than her 'tolerant' counterparts [114.2 (95% CI 76.8, 151.5) months]. The

corresponding value for 'intolerant' subject 11 (82 months) was well within the 95% CI range for 'tolerant' subjects. Analyzing for patients who progressed to visits 2 and 3, the difference in diabetes duration between subject 10 and the lower 95% CI range for thiazolidinedione 'tolerant' subjects decreased to approximately 10 months [166 (subject 10) vs 112.9 (95% CI 69.0, 156.8) (TZD tolerant) months].

Table 2.7 - Demographic characteristics of thiazolidinedione - 'tolerant' and 'intolerant' patients

Subject number/letter by category	*Age (years)*	*Gender*	*Duration of diabetes (months)*
TZD tolerant [a, b]			
1	57	male	24
2	66	female	266
3	62	male	105
4	54	male	65
5	66	female	123
6	65	male	95
7	59	male	113
8	65	male	144
9	59	female	81
A	57	male	77
B	67	female	164
Mean (95% CI) [c]	**61.6 (58.9, 64.2)**		**114.2 (76.8, 151.5)**
TZD intolerant			
10	55	female	166
11	55	female	82

[a] *Subjects 1 to 9 progressed to visit 2;* [b] *subjects A and B were withdrawn after visit 1;* [c] *mean (95% CI) values refer to all participating thiazolidinedione 'tolerant' subjects, irrespective of their progression or otherwise to visit 2.*

2.17.2 Past medical history

Thiazolidinedione tolerant patient number 2 had retinopathy diagnosed 37 months prior to recruitment into the study. Tolerant patients 1 and 8 gave a past history of coronary artery disease. TZD tolerant patient 1 also suffered from peripheral

vascular disease; however none of the patients had undergone any peripheral vascularization procedure or amputation. Tolerant patient A had sustained a cerebrovascular accident in the past. TZD tolerant patient 1 had been diagnosed with C5/C6 radiculopathy. None of the patients gave a history of peripheral neuropathy, autonomic neuropathy or erectile dysfunction. As expected, more than 50% of thiazolidiedione tolerant patients suffered from hypertension (patients 1, 2, 6, 8, A and B), whereas an even higher proportion suffered from dyslipidaemia (patients 1, 2, 3, 5, 7, 8, 9, A, B, and thiazolidinedione intolerant patient number 14). None of the participants were known to suffer from HF at recruitment.

2.17.3 Drug history

Eight out of 11 thiazolidinedione tolerant patients (61.5%) were being treated with pioglitazone at visit 1, with dose ranging from 15 to 45 mg. Daily rosiglitazone dose ranged from 4 to 8 mg. Both intolerant patients were being treated with the lowest possible dose on withdrawal of the offending thiazolidinedione. Duration of thiazolidinedone therapy for 'tolerant' subjects ranged from 7 to 51 months. All participating patients, except one, were being treated with a statin (table 2.8).

Table 2.8 - Oral glucose lowering agent and statin therapy of thiazolidinedione 'tolerant' and 'intolerant' patients.

Subject number/letter by category	*TZD*	*TZD - daily dose prescribed (mg)* [c,d]	*Duration of TZD therapy at visit 1 (months)*	*Metformin daily dose (mg) at visit 1*	*Statin*
TZD tolerant [a, b]					
1	rosiglitazone	4 [c]	11	1500	atorvastatin
2	pioglitazone	30 [c]	51	2500	simvastatin
3	pioglitazone	15 [c]	14	2000	simvastatin
4	pioglitazone	15 [c]	7	2000	atorvastatin
5	rosiglitazone	8 [c]	33	1500	simvastatin
6	pioglitazone	30 [c]	28	1000	
7	pioglitazone	45 [c]	49	2550	simvastatin
8	pioglitazone	30 [c]	33	2700	simvastatin
9	rosiglitazone	8 [c]	42	2000	atorvastatin
A	pioglitazone	30 [c]	30	2000	rosuvastatin
B	pioglitazone	30 [c]	30	1700	atorvastatin
TZD intolerant					
10	rosiglitazone	4 [d]	[e]	2500	simvastatin
11	pioglitazone	15 [d]	[e]	1000	atorvastatin

TZD, thiazolidinedione; [a] subjects 1 to 9 progressed to visit 2; [b] subjects A and B were withdrawn after visit 1; [c] thiazolidinedione dose at visit 1; [d] thiazolidinedione dose at withdrawal; [e] not applicable

2.17.4 Clinical measurements

Table 2.9 summarises the clinical parameters (including anthropometric measurements) of thiazolidinedione 'tolerant' and 'intolerant' patients. All recorded baseline blood pressure readings were generally within the desired range at visit 1 [mean (95% CI) SBP (thiazolidinedione tolerants) = 136.2 (132.2, 140.2) mmHg; mean (95% CI) DBP (thiazolidinedione tolerants) = 75.0 (71.2, 78.8) mmHg]. Patients who did not report symptoms of fluid overload following thiazolidinedione exposure tended to be overweight or obese [mean (95% CI) BMI = 32.54 (30.23, 34.85) kg/m^2]. They were also characterised by an excessive waist circumference

[mean (95% CI) = 113.9 (107.5, 120.3) cm], as outlined in table 2.9. Baseline exploratory data therefore suggested no major differences in baseline body weight, BMI and waist circumference between the tolerant and intolerant groups.

2.17.5 Biochemistry

Patient' glycaemic control was within the range specified by the Protocol (i.e. HbA1c < 9%) at recruitment. Baseline biochemical parameters, namely haematorit, sodium, serum creatinine (and eGFR) and lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides) were also within the Protocol range for most patients, as attested by mean (95% CI) values for thiazolidinedione 'tolerant' subjects. Thiazolidinedione 'intolerant' subject 11 had severe dyslipidaemia. Her LDL-cholesterol concentration could not be determined using the Friedewald equation on account of her triglyceridemia (4.46 mmol/L) (table 2.10).

Table 2.9 - Clinical measurements of thiazolidinedione 'tolerant' and 'intolerant' patients.

Subject number /letter by category	***Mean pulse (beats min^{-1})***	***Mean SBP (mmHg)***	***Mean DBP (mmHg)***	***Height (m)***	***Weight (kg)***	***BMI (kg/m^2)***	***WC (cm)***
TZD tolerant [a, b]							
1	71	124	81	1.68	76.4	27.07	98.0
2	53	139	61	1.60	64.9	25.35	[d]
3	82	127	75	1.80	93.7	28.90	103.0
4	70	143	72	1.65	89.3	32.80	109.5
5	92	130	79	1.60	90.2	35.23	129.5
6	73	145	79	1.72	101.9	34.44	114.5
7	70	137	78	1.74	94.7	31.28	109.0
8	72	136	79	1.80	121.4	37.46	127.5
9	81	135	81	1.60	87.7	34.26	108.0
A	84	140	73	1.73	105.9	35.38	121.0
B	71	141	67	1.57	88.1	35.74	119.0
Mean	**74.5**	**136.2**	**75.0**	**1.68**	**92.2**	**32.54**	**113.9**
(95% CI) [c]	**(68.5, 80.4)**	**(132.2, 140.2)**	**(71.2, 78.8)**	**(1.63, 1.73)**	**(83.5, 100.9)**	**(30.23, 34.85)**	**(107.5, 120.3)**
TZD intolerant							
10	83	141	85	1.57	88.2	35.78	123.0
11	81	127	75	1.60	85.6	33.65	103.5

TZD, thiazolidinedione; WC, waist circumference (cm); [a] *subjects 1 to 9 progressed to visit 2;* [b] *subjects A and B were withdrawn after visit 1*

Table 2.10 - Baseline biochemistry results of thiazolidinedione 'tolerant' and 'intolerant' patients.

Subject number/letter by category	*Haematocrit (%)*	*HbA1c (%)*	*Sodium (mmol/L)*	*Serum creatinine (µmol/L)*	*Total cholesterol (mmol/L)*	*HDL-C (mmol/L)*	*LDL-C (mmol/L)*	*Triglycerides (mmol/L)*
TZD tolerant [a, b]								
1	42.8	7.0	140	69	3.57	0.80	[c]	[c]
2	39.9	6.5	146	64	3.30	1.45	1.45	0.88
3	42.7	8.0	138	66	3.37	1.11	1.27	2.21
4	41.5	8.5	139	86	4.10	1.13	1.90	2.38
5	43.0	8.8	141	51	4.14	0.66	[d]	4.97
6	43.7	6.3	142	76	3.70	0.91	1.94	1.88
7	44.0	7.4	142	76	3.82	1.49	1.81	1.16
8	41.2	8.7	144	94	3.69	1.92	1.45	0.71
9	42.4	7.0	144	61	4.68	1.44	2.33	2.03
A	41.5	7.2	140	55	4.34	1.05	4.30	2.46
B	36.7	6.8	142	63	4.35	1.57	2.32	1.02
Mean	**41.8**	**7.5**	**141.6**	**69.2**	**3.91**	**1.23**	**2.09**	**1.97**
(95% CI) [c]	**(40.6, 43.0)**	**(6.9, 8.0)**	**(140.2, 143.1)**	**(61.6, 76.8)**	**(3.65, 4.17)**	**(1.01, 1.45)**	**(1.50, 2.68)**	**(1.20, 2.74)**
TZD intolerant								
10	37.4	7.5	143	70	4.76	1.65	2.24	1.94
11	[c]	7.3	139	47	8.28	1.47	[c]	4.46

[a] subjects 1 to 9 progressed to visit 2; [b] subjects A and B were withdrawn after visit 1; [c] data unavailable; [d] LDL-C level could not be derived from the Friedewald equation on account of an excessively high serum triglyceride concentration

2.17.6 Sodium exposure - low and high salt diets

Daily morning spot urinary sodium concentrations pertaining to thiazolidinedione 'tolerant' subjects 1 to 9, and 'intolerant' subjects 10 and 11, were measured for five days prior to visit 2 (during which patients followed a moderately low salt diet), and for an additional five days prior to visit 3 (high salt diet). Despite daily variations in urinary sodium excretion, patients were generally compliant to dietary instructions given. Calculation of the area under the curve for thiazolidinedione 'tolerant' and 'intolerant' subjects between days -5 (five days before visit) to 0 (day of visit) (as a surrogate of total dietary sodium exposure) showed that the former increased their urinary sodium excretion by 49 (95% CI 43.5, 59.7)% (vs 20.6% and 125.9% for subjects 10 and 11 respectively) (data not shown).

2.18 Arterial stiffness

Data were accrued from all eleven participating patients [nine thiazolidinedione 'tolerant' (subjects 1 to 9) and two thiazolidinedione 'intolerant' (subjects 10 and 11)] who proceeded to visits two and three. Pulse wave analysis and velocity estimations were carried out once in visit two in patients exposed to a moderately low sodium diet (and before being treated with an intravenous 0.9% saline infusion), and once in visit three following exposure to a high sodium diet. Percentage shift in central augmentation index (cAI), peripheral augmentation index (pAI) and pulse wave velocity (PWV) readings across sodium load categories (chronic high sodium - low sodium) was derived for all participating subjects.

2.18.1 Pulse wave analysis

(i) Central augmentation index

cAI was derived from the ratio of augmentation pressure to pulse pressure. Data are summarised in table 2.11 below. Mean cAI readings for thiazolidinedione 'tolerant' patients exposed to a moderately low sodium diet and a chronic high sodium load were 30.9 (95% CI 25.2, 36.7)% and 30.2 (95% CI 24.0, 36.4)% respectively (table 2.11, figure 2.6). Available data suggest that thiazolidinedione intolerant patients increase their cAI values when exposed to a chronic high sodium load (37.0%, 88.2%), unlike their thiazolidinedione tolerant counterparts [mean (95% CI) = -1.59 (-10.76, 7.58)%], as outlined in table 2.11 and figure 2.7.

Table 2.11 - Central augmentation index (cAI) measurements (%) and derived % differences between sodium load exposures.

Subject number by category	***cAI (low sodium) (%)***	***cAI (chronic high sodium) (%)***	***% difference cAI (chronic high sodium - low sodium)***
TZD tolerant			
1	30	30	0
2	46	49	6.5
3	16	20	25.0
4	21	18	-14.3
5	38	36	-5.3
6	31	33	6.5
7	29	22	-24.1
8	32	32	0
9	35	32	-8.6
Mean (95% CI)	**30.9 (25.2, 36.70)**	**30.2 (24.0, 36.4)**	**-1.59 (-10.76, 7.58)**
TZD intolerant			
10	27	37	37.0
11	17	32	88.2

Figure 2.6 – Mean (95% CI) central augmentation index (cAI) values (%) for thiazolidinedione (TZD) tolerant (n = 9, plotted in blue) and individual cAI readings for thiazolidinedione intolerant (n = 2, plotted in red) subjects exposed to a low sodium diet and a chronic high sodium load.

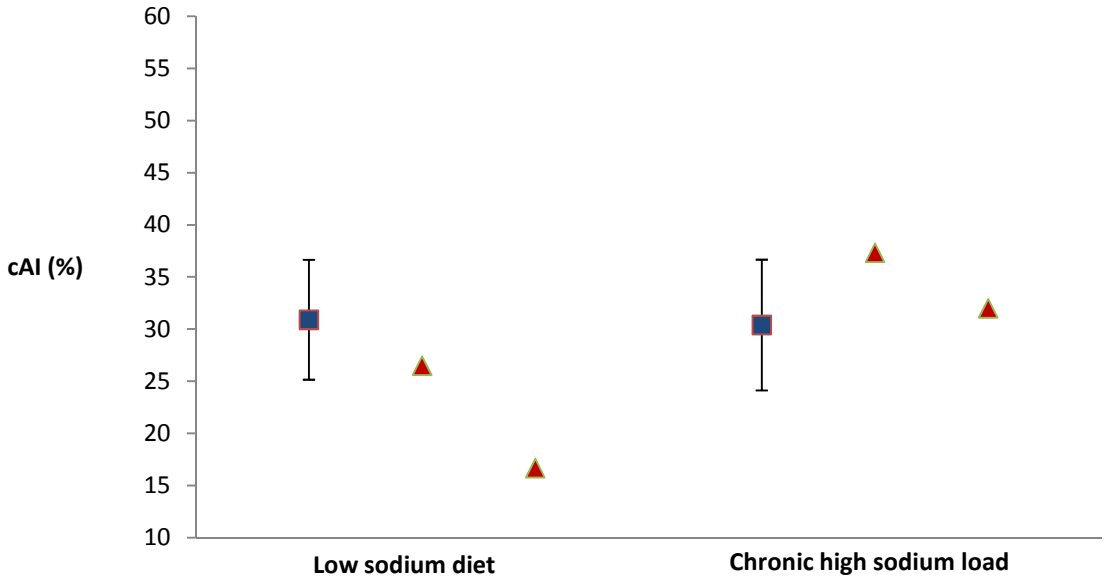
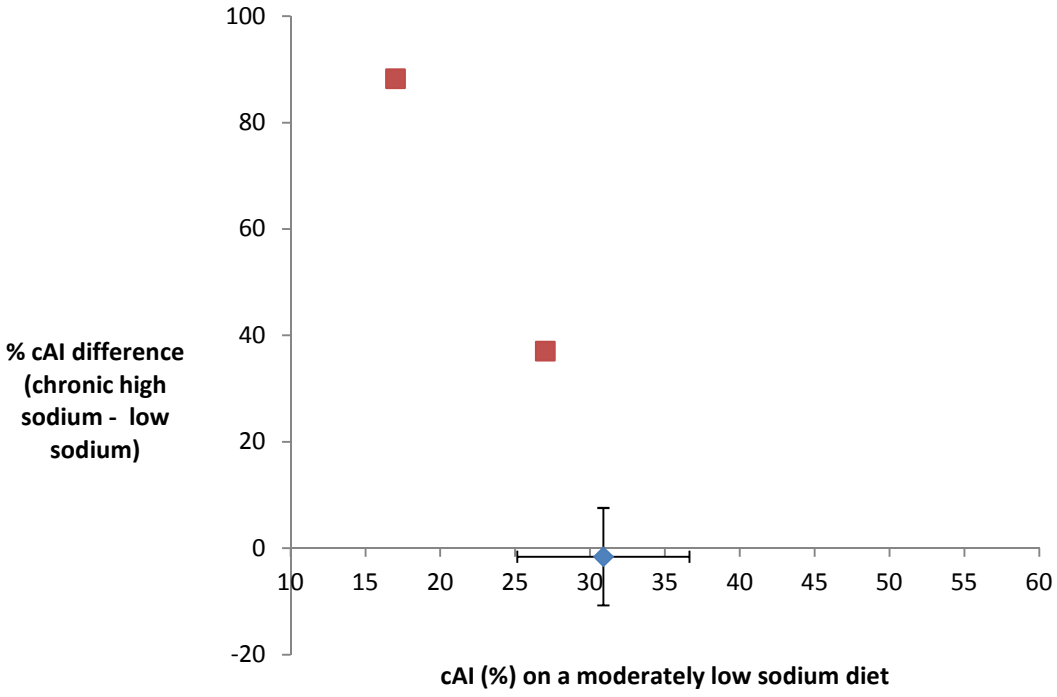


Figure 2.7 – Percentage difference in central augmentation index (cAI) readings (%) between exposure to a moderately low sodium diet and a chronic high sodium load for thiazolidinedione (TZD) tolerant (n = 9, plotted in blue) and TZD intolerant (n = 2, plotted in red) subjects. Mean and 95% confidence intervals were derived for TZD tolerant subjects.



(ii) Peripheral augmentation index

Peripheral augmentation index (pAI) was likewise derived using applanation tonometry from the ratio of late systolic pressure (P2) to early systolic pressure (P1). Percentage shifts in pAI readings were also estimated for visits 2 and 3, as outlined in table 2.12.

Table 2.12 - Peripheral augmentation index (pAI) measurements (%) and derived % differences between sodium load exposures.

Subject number by category	*pAI (low sodium) (%)*	*pAI (chronic high sodium) (%)*	*% difference pAI (chronic high sodium - low sodium)*
TZD tolerant			
1	143	142	-0.7
2	185	196	5.9
3	119	126	5.0
4	127	122	-3.9
5	162	156	-3.7
6	145	150	3.4
7	141	129	-8.5
8	146	148	0.7
9	153	148	-3.9
Mean	**147.1**	**146.3**	**-0.6**
(95% CI)	**(134.7, 159.5)**	**(131.9, 160.7)**	**(-3.74, 2.54)**
TZD intolerant			
10	136	159	16.9
11	120	147	22.5

Mean pAI readings for thiazolidinedione tolerant patients were 147.1 (95% CI 134.7, 159.5) % and 146.3 (95% CI 131.9, 160.7)% under moderately low and high sodium dietary conditions respectively (table 2.12, figure 2.8). Oedema prone TZD patients seemingly increase their pAI when subjected to a chronic sodium load, unlike their thiazolidinedione tolerant counterparts [-0.6 (95% CI -3.74, 2.54)% (TZD tolerant) vs 16.9%, 22.5% (TZD intolerant)] (table 2.12, figure 2.9).

Figure 2.8 – Mean (95% CI) peripheral augmentation index (pAI) values (%) for thiazolidinedione (TZD) tolerant (n = 9, plotted in blue) and individual pAI readings for thiazolidinedione intolerant (n = 2, plotted in red) subjects exposed to a low sodium diet and a chronic high sodium load.

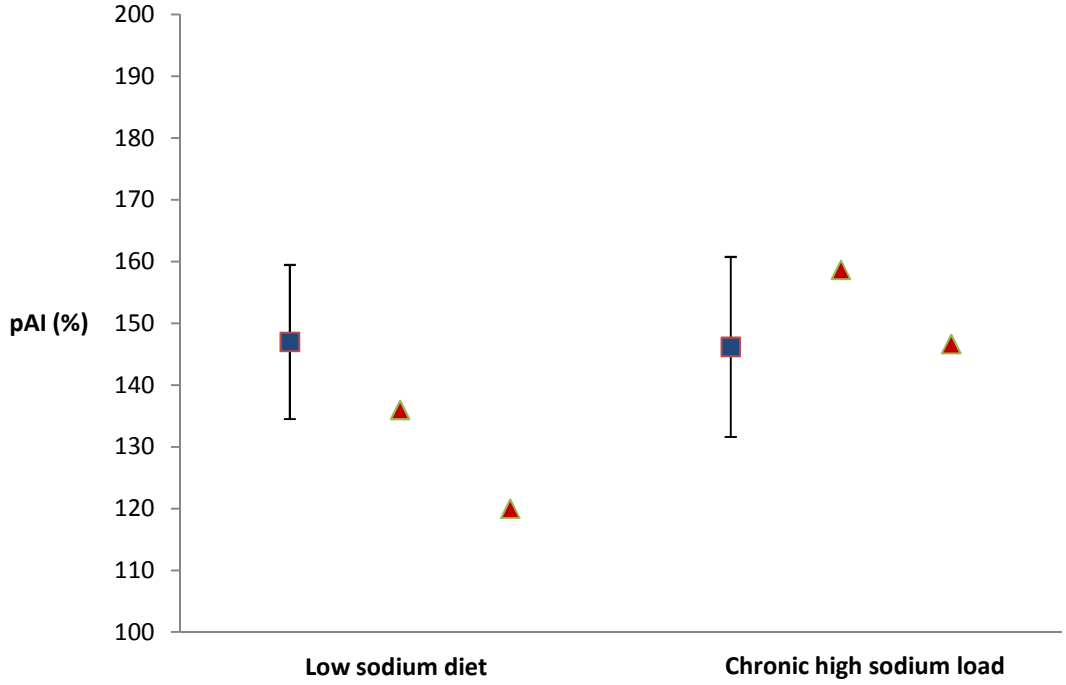
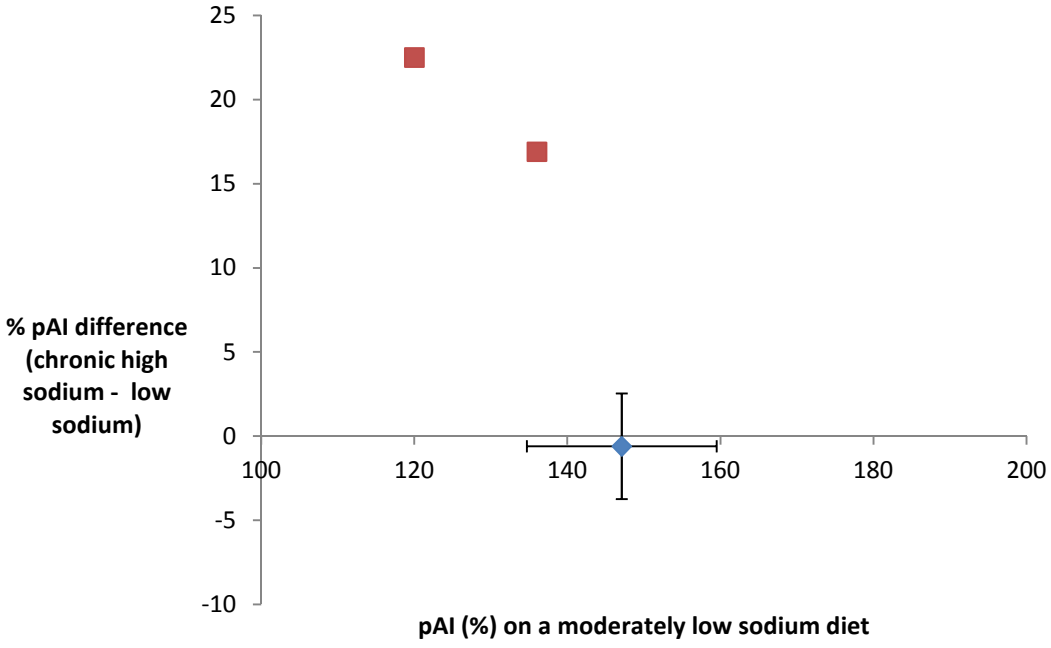


Figure 2.9 – Percentage difference in peripheral augmentation index (pAI) readings (%) between exposure to a moderately low sodium diet and a chronic high sodium load for thiazolidinedione (TZD) tolerant (n = 9, plotted in blue) and TZD intolerant (n = 2, plotted in red) subjects. Mean and 95% confidence intervals were derived for TZD tolerant subjects.



2.18.2 Pulse wave velocity

Pulse wave velocity data pertaining to the participating subjects are summarised in table 2.13 below. Mean (95% CI) PWV readings for thiazolidinedione 'tolerant' patients exposed to a moderately low sodium diet and a chronic high sodium load were 8.57 (7.84, 9.30) m/s and 8.32 (7.61, 9.03) m/s respectively. Available data do not suggest any differences in baseline PWV between oedema prone and thiazolidinedione tolerant patients, irrespective of sodium exposure. Likewise, there seems to be no appreciable difference in % PWV shift across sodium load exposures between the two groups [-2.82 (95% CI -5.34, -0.30)% (TZD tolerant) vs -9.4%, 11.4% (TZD intolerant)], as outlined in table 2.13.

Table 2.13 - Pulse wave velocity (PWV) measurements (m/s) and derived % differences between sodium load exposures.

Subject number by category	*PWV (low sodium) (m/s)*	*PWV (chronic high sodium) (m/s)*	*% difference PWV (chronic high sodium - low sodium)*
TZD tolerant			
1	10.6	9.9	-6.6
2	8.0	7.6	-5.0
3	9.7	9.2	-5.2
4	8.7	9.2	5.7
5	7.0	6.6	-5.7
6	7.7	7.6	-1.3
7	8.2	8.1	-1.2
8	9.3	9.2	-1.1
9	7.9	7.5	-5.1
Mean (95% CI)	**8.57 (7.84, 9.30)**	**8.32 (7.61, 9.03)**	**-2.82 (-5.34, -0.30)**
TZD intolerant			
10	8.5	7.7	-9.4
11	7.9	8.8	11.4

2.19 Echocardiography

Echocardiographic parameters (left ventricular ejection fraction, E/A ratio, E prime, E/e prime ratio, left ventricular mass) were captured for participating subjects in visits two and three. Data were collected for nine, eight and nine thiazolidinedione tolerant subjects following exposure to a moderately low, acute high and chronic high sodium loads respectively (one patient declined an echo following intravenous saline administration). Data were captured from both TZD 'intolerant' patients in all three instances. Preliminary exploratory data suggests no differences in any of the measured echocardiographic parameters between thiazolidinedione patient categories. Plotting percentage change in any of these measurements across sodium load categories for both TZD tolerant and intolerant patients did not yield any consistent trends (appendix tables II.1 to II.5).

2.20 Biomarkers

In a bid to identify predisposing factors for thiazolidinedinoe-associated fluid retention, this study measured a number of biomarkers of interest, as outlined in section I. Plasma samples were collected to measure vascular endothelial growth factor (VEGF), ANP, BNP, N-terminal prohormone of B-type natriuretic peptide (NT-pro-BNP), aldosterone, renin and copeptin for each patient at visit 2 (moderately low sodium diet, *before* infusion of intravenous saline) and visit 3 (chronic sodium load). This enabled a relative comparison between thiazolidinedione 'tolerant' and 'intolerant' subjects.

2.20.1 Vascular endothelial growth factor

Plasma was sampled for VEGF level estimation from eight and nine thiazolidinedione tolerant patients at visits 2 and 3 respectively, and from both thiazolidinedione intolerant patients at either visit. Mean (95% CI) VEGF readings for 'tolerant' subjects were 57.3 (12.0, 102.6) pg/mL and 38.6 (24.1, 53.1) pg/mL after exposure to a moderately low and a chronic high sodium load respectively (appendix table II.6). Generally decreasing for 'tolerant' patients on sodium loading, available data suggest no significant difference in VEGF levels between thiazolidinedione categories on exposure to a low sodium diet. However, exposure to a moderately high sodium diet for five days resulted in seemingly lower VEGF levels for thiazolidinedione intolerant subjects compared to their intolerant counterparts (appendix table II.6). Plotting percentage change in VEGF readings between exposure to a moderately low sodium diet and a chronic high sodium load showed a mean (95% CI) VEGF reduction of 11.6 (-32.5, 9.3)% for thiazolidinedione tolerant individuals. The two intolerant subjects exhibited VEGF changes on either side of the 95% CI range for their tolerant counterparts (appendix table II.6).

2.20.2 Atrial natriuretic peptide

ANP data were available from eight and seven thiazolidinedione tolerant patients, and from both intolerant subjects after exposure to a moderately low and a chronic high sodium load respectively. Mean (95% CI) ANP readings for TZD tolerant patients were 40.33 (7.37, 73.29) fmol/mL and 21.3 (9.45, 33.15) fmol/mL respectively. No significant difference in ANP levels between either thiazolidinedione category, irrespective of sodium exposure (table 2.14, figure 2.10) was detected. However, plots of percentage change in ANP between the period of low and high sodium diets suggests an 80-129% increase for TZD intolerant patients compared with a mean (95% CI) 5.2% increase (-53.4, 63.8) for thiazolidinedione tolerant patients (table 2.14, figure 2.11).

Table 2.14 - Atrial natriuretic peptide (ANP) measurements (fmol/mL) and derived % differences between sodium load exposures for visits 2 and 3

Subject number by category	*ANP (fmol/mL) (low sodium)*	*ANP (fmol/mL) (chronic high sodium)*	*% difference ANP (chronic high sodium - low sodium)*
TZD tolerant			
1	21.162	16.506	-22.0
2	26.289	43.736	66.4
3	65.473	8.938	-86.3
4	5.4796	12.298	124.4
5	1.999	0.134	-93.3
6	26.022	30.531	17.3
7	28.152	36.659	30.2
8	148.033	[a]	[c]
9	[b]	[b]	[c]
Mean	**40.330**	**21.300**	**5.2**
(95% CI)	**(7.370, 73.290)**	**(9.450, 33.150)**	**(-53.4, 63.8)**
TZD intolerant			
10	25.726	46.297	80.0
11	10.815	24.802	129.3

[a] haemolyzed sample, rendering result dubious; [b] patient's ANP data are unavailable; [c] derivation of % difference not possible due to missing data

Figure 2.10 – Mean (95% CI) atrial natriuretic peptide (ANP) values (fmol/mL) for thiazolidinedione (TZD) tolerant [n = 8 (low sodium diet), n = 7 (chronic high sodium load) plotted in blue] and individual ANP readings for thiazolidinedione intolerant (n = 2, plotted in red) subjects exposed to a low sodium diet and a chronic high sodium load.

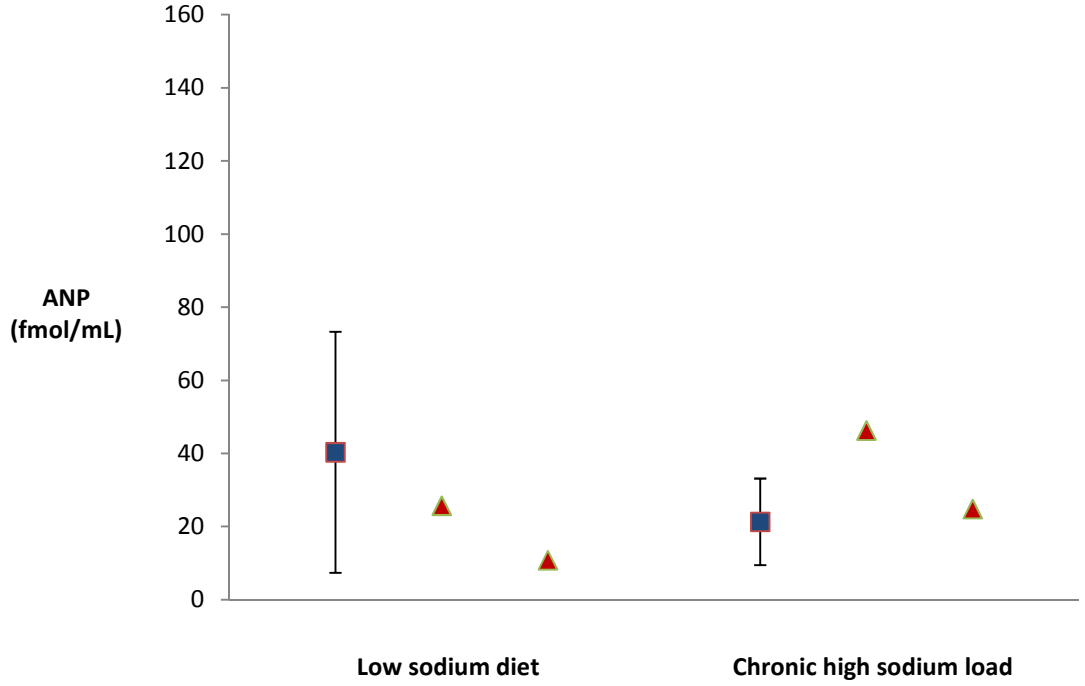
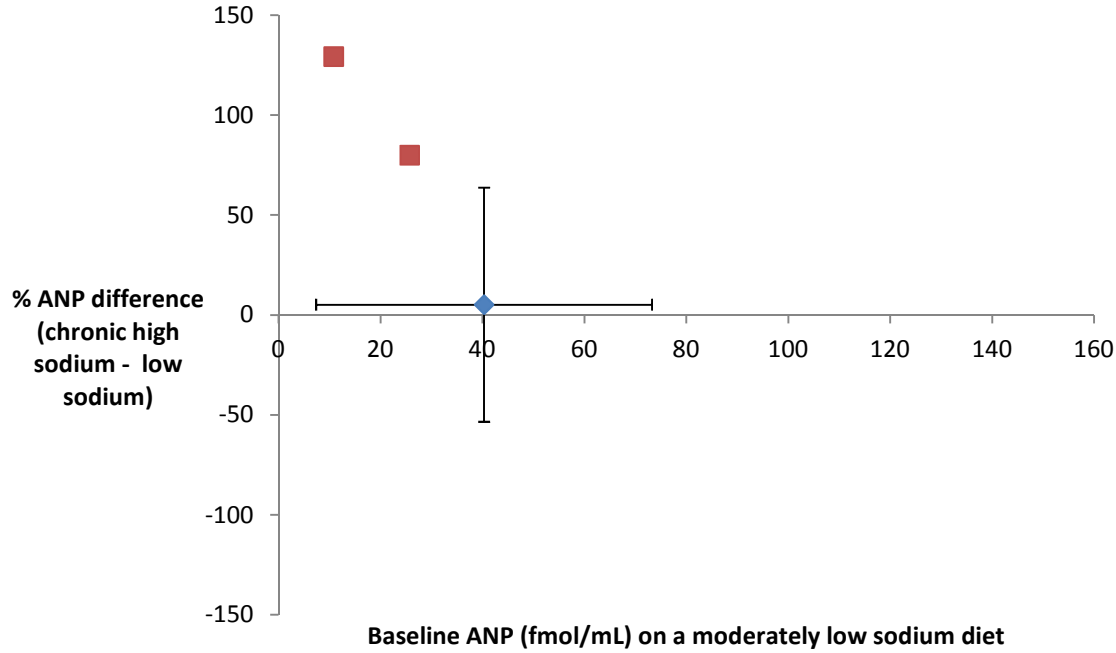


Figure 2.11 – Percentage difference in atrial natriuretic peptide (ANP) readings (fmol/mL) between exposure to a moderately low sodium diet and a chronic high sodium load for thiazolidinedione (TZD) tolerant [n = 7 (low sodium diet), n = 8 (chronic high sodium load) plotted in blue] and intolerant (n = 2, plotted in red) subjects. Mean and 95% confidence intervals were derived for thiazolidinedione tolerant subjects.



2.20.3 B-type natriuretic peptide

BNP concentrations were measured in eight thiazolidinedione tolerant and both TZD intolerant patients in each of visits two and three. Mean (95% CI) BNP levels decreased for the oedema free subjects on sodium exposure [16.87 (1.08, 32.66) pg/mL (low sodium) vs 8.50 (5.53, 11.47) pg/mL (high sodium)] (table 2.15, figure 2.12). Exploratory data suggest no significant difference in BNP levels between thiazolidinedione categories after exposure to a low sodium diet. Individually plotted data for oedema prone subjects lie beyond, albeit on either side, of the mean (95% CI) reference range for thiazolidinedione 'tolerant' subjects on exposure to a chronic moderately high sodium load (figure 2.12). The latter subgroup were characterised by a mean (95% CI) 27.5% increase (-37.22, 92.22) in prevailing BNP concentrations on progressing from a moderately low to a moderately high sodium diet. TZD intolerant subjects tended to exhibit a greater increase (90.1% and 249.7% respectively). However, any conclusions are rendered dubious by the observation that the 90.1% increase reported for one of the oedema prone patients marginally overlaps with the upper limit of the 95% CI reference range for tolerant subjects (table 2.15, figure 2.13).

Table 2.15 - B-type natriuretic peptide (BNP) measurements (pg/mL) and derived % differences between sodium load exposures for visits 2 and 3

Subject number by category	*BNP (pg/mL) (low sodium)*	*BNP (pg/mL) (chronic high sodium)*	*% difference BNP (chronic high sodium - low sodium)*
TZD tolerant			
1	5.284	9.399	77.9
2	4.645	11.082	138.6
3	3.511	4.610	31.3
4	29.089	4.687	-83.9
5	68.905	12.482	-81.9
6	1.975	5.138	160.2
7	7.656	4.917	-35.8
8	13.868	15.697	13.2
9	[a]	[a]	[b]
Mean	**16.870**	**8.500**	**27.5**
(95% CI)	**(1.080, 32.660)**	**(5.530, 11.470)**	**(-37.22, 92.22)**
TZD intolerant			
10	2.303	4.379	90.1
11	6.440	22.523	249.7

[a] patient's BNP data were unavailable; [b] calculation not possible due to missing data

Figure 2.12 – Mean (95% CI) B-type natriuretic peptide (BNP) values (pg/mL) for thiazolidinedione (TZD) tolerant (n = 8, plotted in blue) and individual BNP readings for thiazolidinedione intolerant (n = 2, plotted in red) subjects exposed to a low sodium diet and a chronic high sodium load.

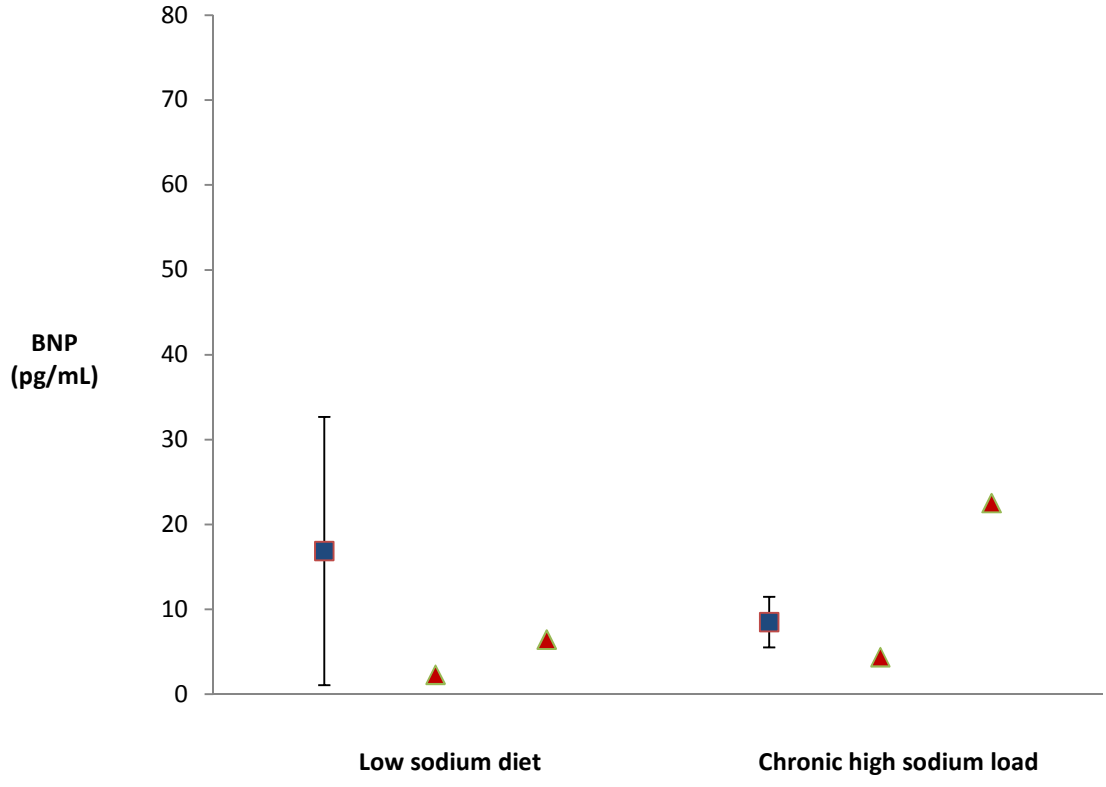
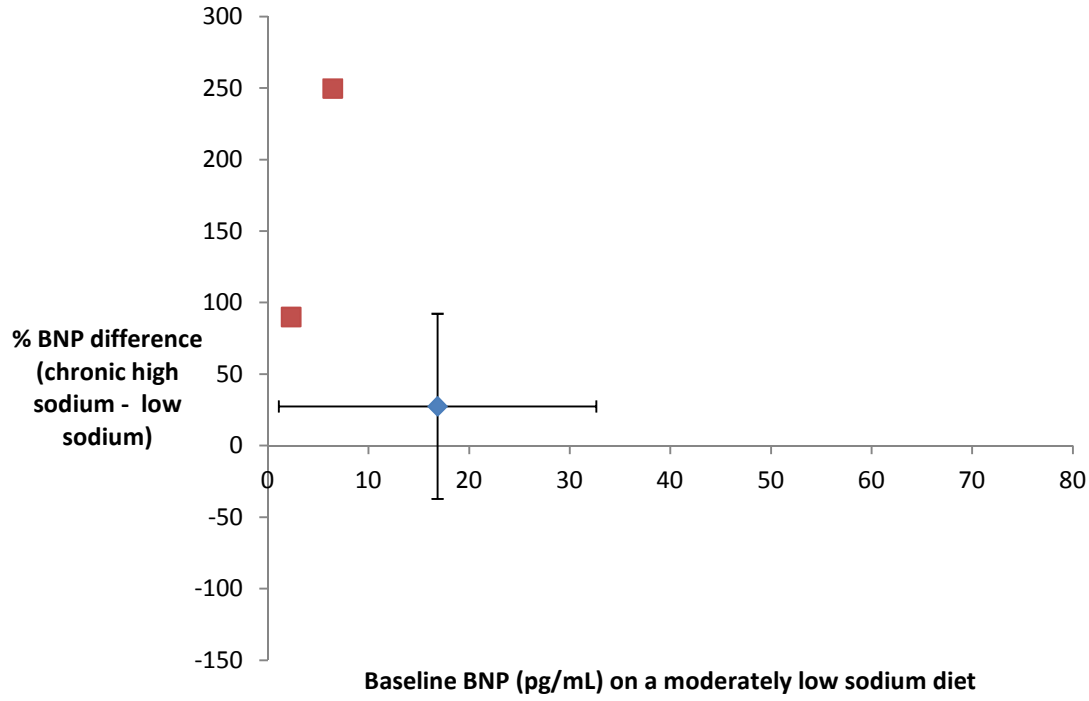


Figure 2.13 – Percentage difference in B-type natriuretic peptide (BNP) readings (pg/mL) between exposure to a moderately low sodium diet and a chronic high sodium load for thiazolidinedione (TZD) tolerant (n = 8, plotted in blue) and intolerant (n = 2, plotted in red) subjects. Mean and 95% confidence intervals were derived for thiazolidinedione tolerant subjects



2.20.4 N-terminal prohormone of B-type natriuretic peptide

N-terminal prohormone of B-type natriuretic peptide (NT-pro-BNP) was measured in eight thiazolidinedione 'tolerant' subjects [mean (95% CI) 440.29 (347.05, 533.53) fmol/mL (low sodium); 501.1 (355.08, 647.12) fmol/mL (high sodium)]. Plotting individual data points for TZD intolerant patients suggest no significant difference between either thiazolidinedione category at either visit (table 2.16). In a similar vein, thiazolidinedione tolerant patients were characterised by a mean (95% CI) 14.8 % increase (-21.86, 51.46) in prevailing NT-pro-BNP (vs 4.4% and 31.8% increase for TZD intolerant ones) (table 2.16).

Table 2.16 - N-terminal prohormone of B-type natriuretic peptide (NT-pro-BNP) measurements (fmol/mL) and derived % differences between sodium load exposures for visits 2 and 3.

Subject number by category	*NT-pro-BNP (fmol/mL) (low sodium)*	*NT-pro-BNP (fmol/mL) (chronic high sodium)*	*% difference NT-pro-BNP (chronic high sodium - low sodium)*
TZD tolerant			
1	377.184	300.216	-20.4
2	379.128	453.702	19.7
3	471.018	981.57	108.4
4	759.132	293.82	-61.3
5	359.022	586.938	63.5
6	411.786	359.736	-12.6
7	348.504	446.826	28.2
8	416.568	386.472	-7.2
9	[a]	700.788	[b]
Mean	**440.29**	**501.1**	**14.8**
(95% CI)	**(347.05, 533.53)**	**(355.08, 647.12)**	**(-21.86, 51.46)**
TZD intolerant			
10	535.656	559.014	4.4
11	344.214	453.666	31.8

[a] patient's NT-pro-BNP data were unavailable; [b] estimation of % difference not possible due to missing data

2.20.5 Aldosterone

As expected, plasma aldosterone concentrations decreased in response to chronic salt loading for either thiazolidinedione category. Mean (95% CI) values for thiazolidinedione tolerant subjects were 292.59 (155.87, 429.31) pg/mL and 99.4 (22.77, 176.03) pg/mL after exposure to a moderately low and a chronic high sodium load respectively (table 2.17, figure 2.14). Exploratory data suggest no significant difference in plasma aldosterone readings between the two thiazolidinedione categories (figure 2.14). While thiazolidinedione intolerant subjects generally exhibited a greater reduction in prevailing plasma aldosterone concentrations on salt loading (-69.7% and -86.2% respectively), these values overlap with the lower end-

point of the 95% CI for tolerant subjects [mean (95% CI) = -50.9 (-87.07, -14.73)%] (table 2.17, figure 2.15).

Table 2.17 - Plasma aldosterone measurements (pg/mL) and derived % differences between sodium load exposures for visits 2 and 3

Subject number by category	*Aldosterone (pg/mL) (low sodium)*	*Aldosterone (pg/mL) (chronic high sodium)*	*% difference aldosterone (chronic high sodium - low sodium)*
TZD tolerant			
1	421.018	32.506	-92.3
2	199.085	110.64	-44.4
3	194.593	20.289	-89.6
4	93.108	154.133	65.5
5	699.726	386.860	-44.7
6	127.101	81.291	-36.0
7	255.647	50.408	-80.3
8	350.456	52.320	-85.1
9	[a]	6.447	[b]
Mean (95% CI)	**292.590 (155.870, 429.310)**	**99.400 (22.770, 176.030)**	**-50.9 (-87.07, -14.73)**
TZD intolerant			
10	108.970	32.967	-69.7
11	358.130	49.375	-86.2

[a] Patient's plasma aldosterone data unavailable; [b]derivation of % difference not possible due to missing data

Figure 2.14 – Mean (95% CI) plasma aldosterone values (pg/mL) for thiazolidinedione (TZD) tolerant [n = 8 (low sodium diet), n = 9 (chronic high sodium load) plotted in blue] and individual aldosterone readings for thiazolidinedione intolerant (n = 2, plotted in red) subjects exposed to a low sodium diet and a chronic high sodium load.

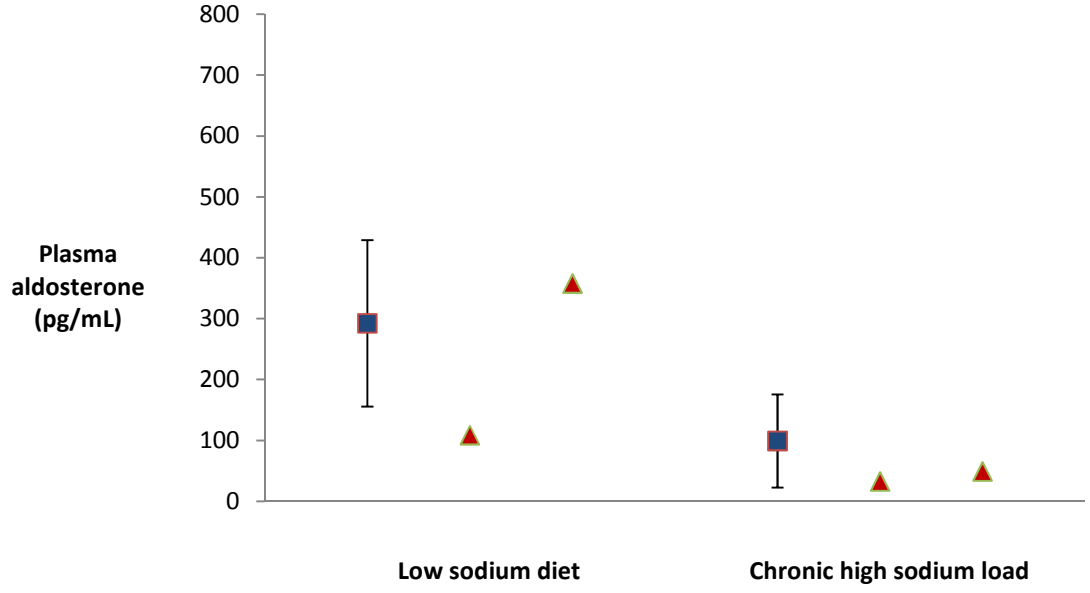
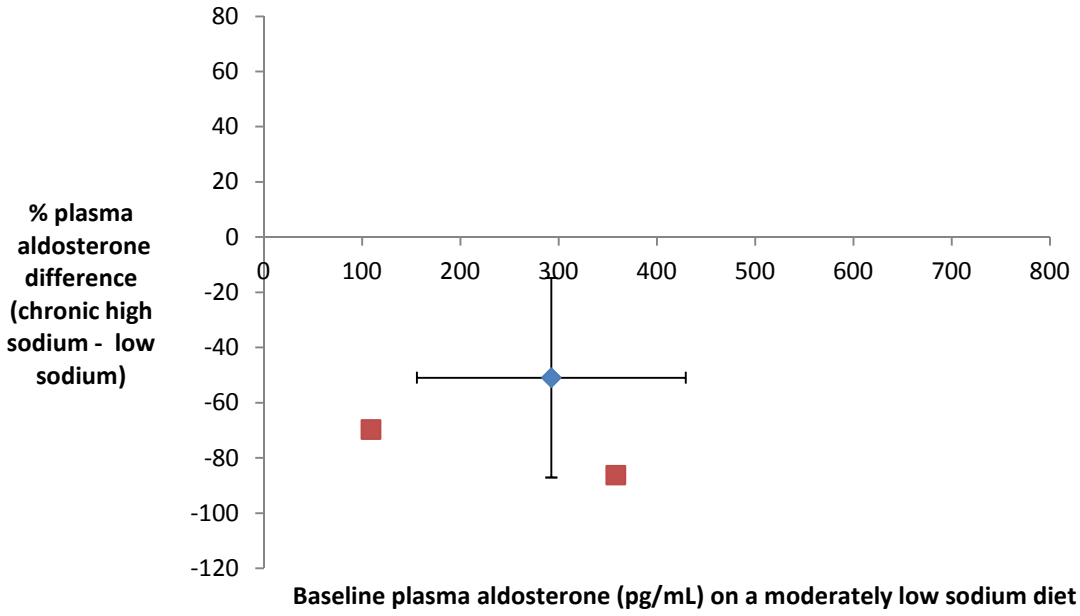


Figure 2.15 – Percentage difference in plasma aldosterone readings (pg/mL) between exposure to a moderately low sodium diet and a chronic high sodium load for thiazolidinedione (TZD) tolerant [n = 8 (low sodium diet), n = 9 (chronic high sodium load) plotted in blue] and intolerant (n = 2, plotted in red) subjects. Mean and 95% confidence intervals were derived for thiazolidinedione tolerant subjects



2.20.6 Renin

Plasma renin levels were measured in eight and nine thiazolidinedione tolerant patients, and in both TZD intolerant subjects at visits 2 and 3 respectively. In concordance with earlier reported aldosterone results, exposure to a chronic sodium load was associated with a reduction in prevailing renin concentrations in either thiazolidinedione category [mean (95% CI) = -71.2 (-82.5, -59.9) % for thiazolidinedione tolerant subjects vs -86.2% and -99.9% (thiazolidinedione intolerant)] (table 2.18, figure 2.17). Thus, this preliminary data suggest that patients prone to thiazolidinedione-induced oedema decrease their prevalent plasma renin by a greater margin than their tolerant counterparts. Plots of individual plasma renin readings for intolerant subjects at either visit and comparing these to the mean (95%

CI) values for their TZD tolerant counterparts suggests no significant differences between either patient category (table 2.18, figure 2.16).

Table 2.18 - Plasma renin measurements (ng/mL/hour) and derived % differences between sodium load exposures for visits 2 and 3

Subject number by category	*Renin (ng/mL/hour) (low sodium)*	*Renin (ng/mL/hour) (chronic high sodium)*	*% difference renin (chronic high sodium - low sodium)*
TZD tolerant			
1	3.234	0.269	-91.7
2	0.314	0.134	-57.3
3	1.798	0.262	-85.4
4	0.468	0.191	-59.2
5	3.644	1.946	-46.6
6	0.281	0.053	-81.1
7	1.644	0.263	-84.0
8	1.860	0.659	-64.6
9	[a]	0.062	[b]
Mean	**1.655**	**0.427**	**-71.2**
(95% CI)	**(0.76, 2.55)**	**(0.04, 0.82)**	**(-82.5, -59.9)**
TZD intolerant			
10	1.649	0.227	-86.2
11	1.767	0.001	-99.9

[a] patient's plasma renin data were unavailable; [b] estimation of % difference not possible due to missing data

Figure 2.16 – Mean (95% CI) plasma renin values (ng/mL/hour) for thiazolidinedione (TZD) tolerant [n = 8 (low sodium diet), n = 9 (chronic high sodium load) plotted in blue] and individual renin readings for thiazolidinedione intolerant (n = 2, plotted in red) subjects exposed to a low sodium diet and a chronic high sodium load.

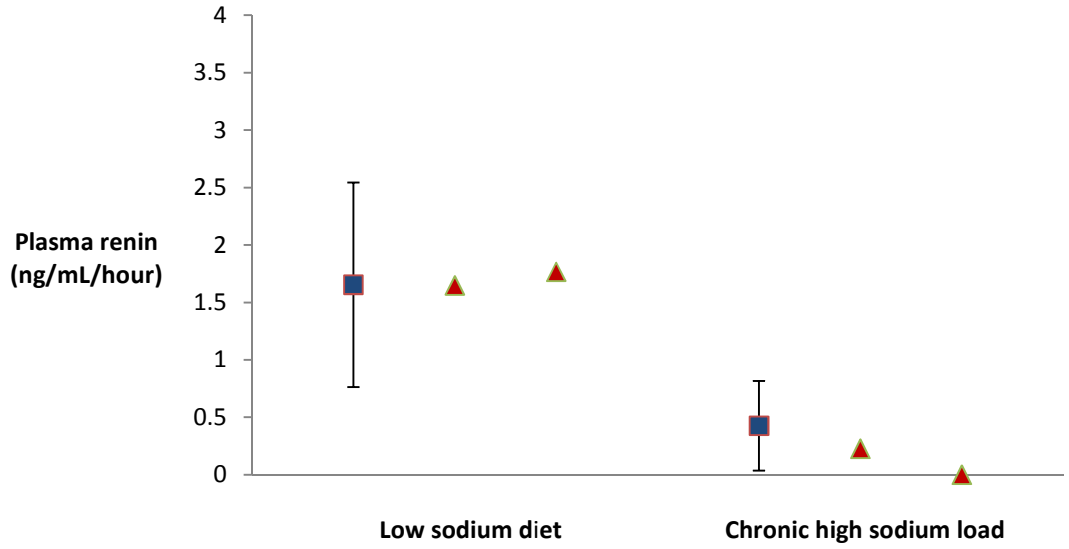
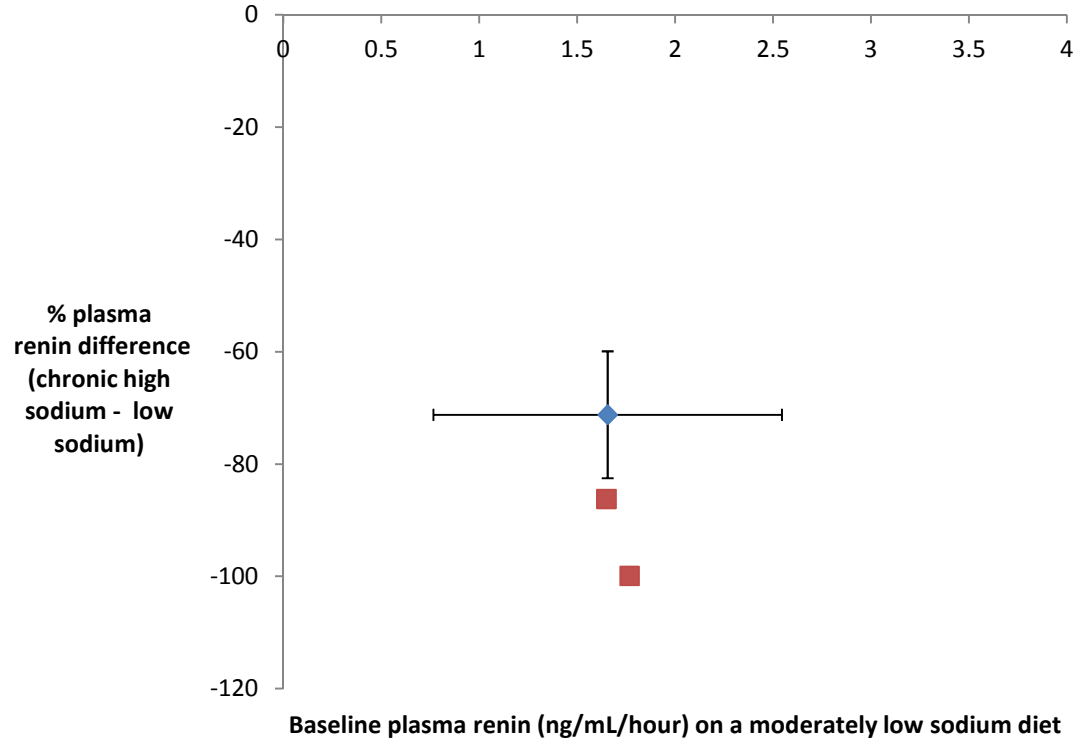


Figure 2.17 – Percentage difference in plasma renin readings (ng/mL/hour) between exposure to a moderately low sodium diet and a chronic high sodium load for thiazolidinedione (TZD) tolerant [n = 8 (low sodium diet), n = 9 (chronic high sodium load) plotted in blue] and intolerant (n = 2, plotted in red) subjects. Mean and 95% confidence intervals were derived for thiazolidinedione tolerant subjects



2.20.7 Copeptin

Copeptin was measured using a highly sensitive assay from eight and nine thiazolidinedione 'tolerant' patients at visits 2 and 3 respectively, and from both thiazolidinedione 'intolerant' subjects at either visit. Mean (95% CI) copeptin readings decreased for such patients when progressing from a moderately low sodium to a chronic high sodium load [5.83 (3.73, 7.93) (low sodium) vs 4.1 (3.19, 5.01) (high sodium)] (appendix table II.7). Individual readings for thiazolidinedione 'intolerant' subjects stood beyond, albeit on either side, of the 95% CI range for their 'oedema free' counterparts (appendix table II.7). Thiazolidinedione 'tolerant' subjects experienced a mean (95% CI) copeptin reduction of 17.2% (-40.87, 5.67) on chronic salt loading. Plots did not suggest that differences from TZD intolerant counterparts (-30.4% and 7.5% respectively) (appendix table II.7).

2.21 Haematocrit shifts in response to salt loading

Consistent with the observations on urinary sodium excretion, both thiazolidinedione tolerant and intolerant patients exhibited a decrease in their haematocrit (i.e. haemodiluted) in response to salt loading, whether acute or chronic (table 2.19, figure 2.18). Thiazolidinedione intolerant subjects 10 and 11 were characterised by lower haematocrit values at all three salt loading states (table 2.19, figure 2.18). The degree of reduction (expressed as a percentage) for thiazolidinedione tolerant subjects tended to be greater following acute compared with chronic salt loading (table 2.19, figures 2.19 and 2.20). Comparing % change in haematocrit across thiazolidinedione categories, both intolerant subjects had a larger decrease in

haematocrit following exposure to a five day high sodium diet [-6.99%, -7.54% respectively vs mean (95% CI) values of -3.69 (-5.89, -1.49) % for 'tolerant' patients] (table 2.19, figure 2.20). Analyzing for the percentage difference across low and acute high salt loading, TZD intolerant patient 10 had a numerically larger decrease in haematocrit (-5.52%) compared with her thiazolidinedione tolerant counterparts [mean (95% CI) = -4.13 (-5.29, -2.97) %], but this was not the case for subject 11 (table 2.19, figure 2.19).

Table 2.19 - Haematocrit measurements (%) and derived % differences between sodium load exposures for visits 2 and 3

Subject number by category	*Haematocrit (low Na) (%)*	*Haematocrit (acute high Na) (%)*	*Haematocrit (chronic high Na) (%)*	*% difference haematocrit (acute high Na - low sodium)*	*% difference haematocrit (chronic high Na - low sodium)*
TZD tolerant					
1	44.40	[a]	42.45	[b]	-4.39
2	38.88	36.35	36.68	-6.50	-5.66
3	42.05	40.45	41.13	-3.80	-2.20
4	39.15	37.45	39.58	-4.34	1.09
5	43.00	40.45	41.43	-5.93	-3.66
6	45.18	44.00	40.78	-2.60	-9.74
7	44.45	43.15	43.75	-2.92	-1.57
8	41.00	39.85	39.60	-2.80	-3.41
9	[a]	[a]	38.95	[b]	[b]
Mean (95% CI)	**42.26 (40.58, 43.94)**	**40.24 (38.20, 42.28)**	**40.48 (39.13, 41.83)**	**-4.13 (-5.29, -2.97)**	**-3.69 (-5.89, -1.49)**
TZD intolerant					
10	37.58	35.50	34.95	-5.52	-6.99
11	39.48	37.70	36.50	-4.50	-7.54

[a] *data unavailable;* [b] *calculation not possible due to missing data*

Figure 2.18 – Mean (95% CI) haematocrit readings (%) for thiazolidinedione (TZD) tolerant [n = 8 (low sodium diet); n = 7 (acute high sodium load); n = 9 (chronic high sodium diet), plotted in blue] and individual haematocrit readings for thiazolidinedione intolerant (n = 2, plotted in red) subjects exposed to a low sodium diet, an acute high sodium load and a chronic high sodium load.

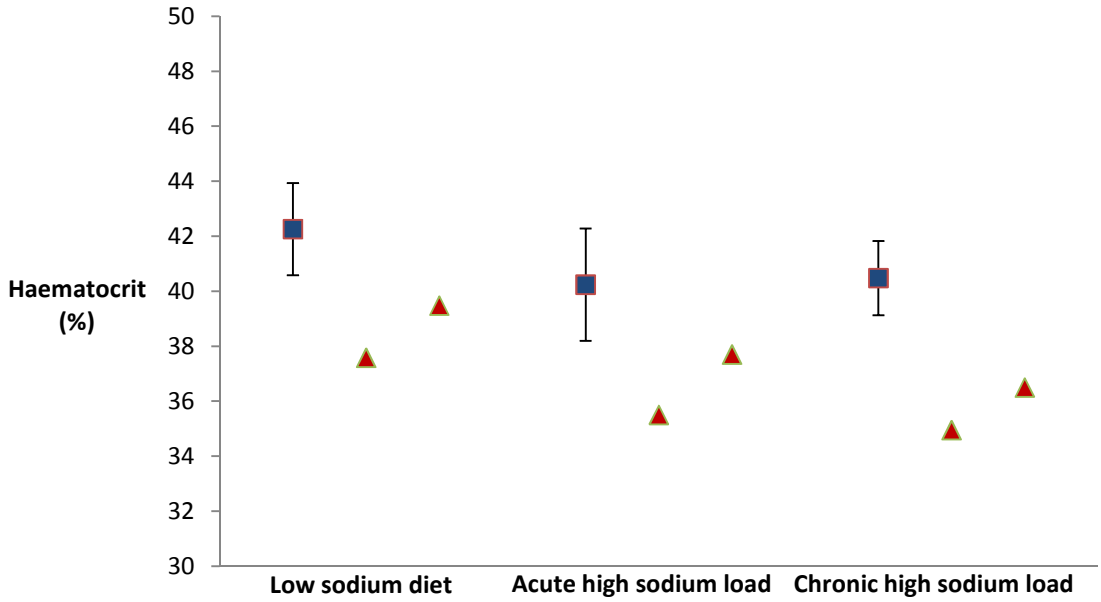


Figure 2.19 – Percentage difference in haematocrit between exposure to a moderately low sodium diet and an acute high sodium load for thiazolidinedione (TZD) tolerant [n = 8 (low sodium), n = 7 (acute high sodium), plotted in blue] and intolerant (n = 2, plotted in red) subjects. Mean and 95% confidence intervals were derived for TZD tolerant subjects.

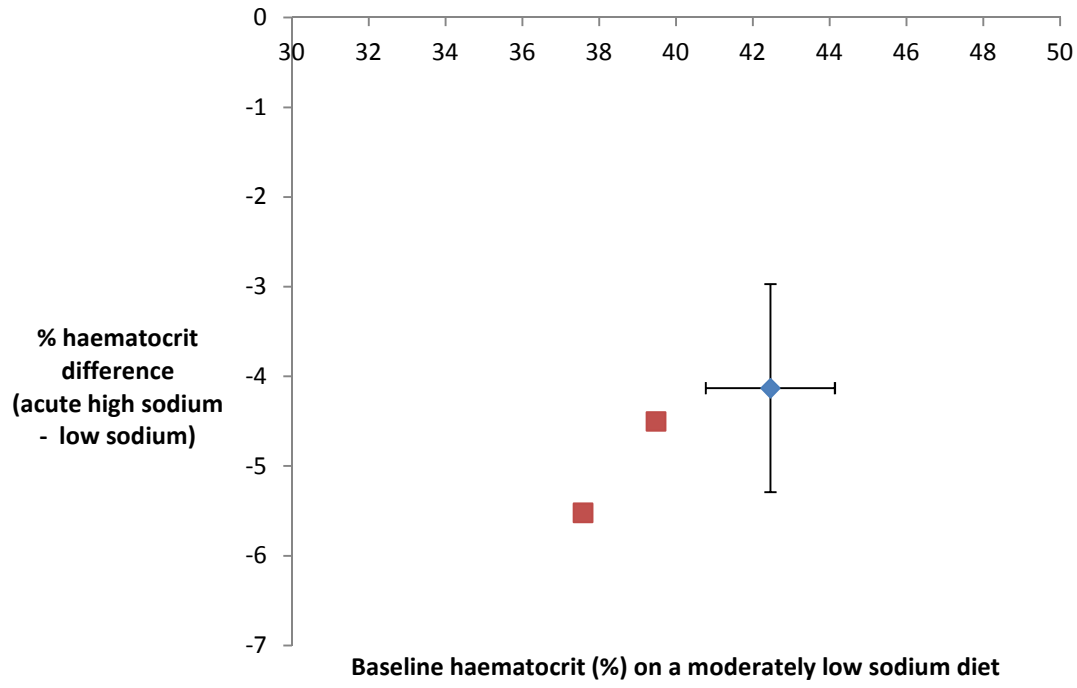
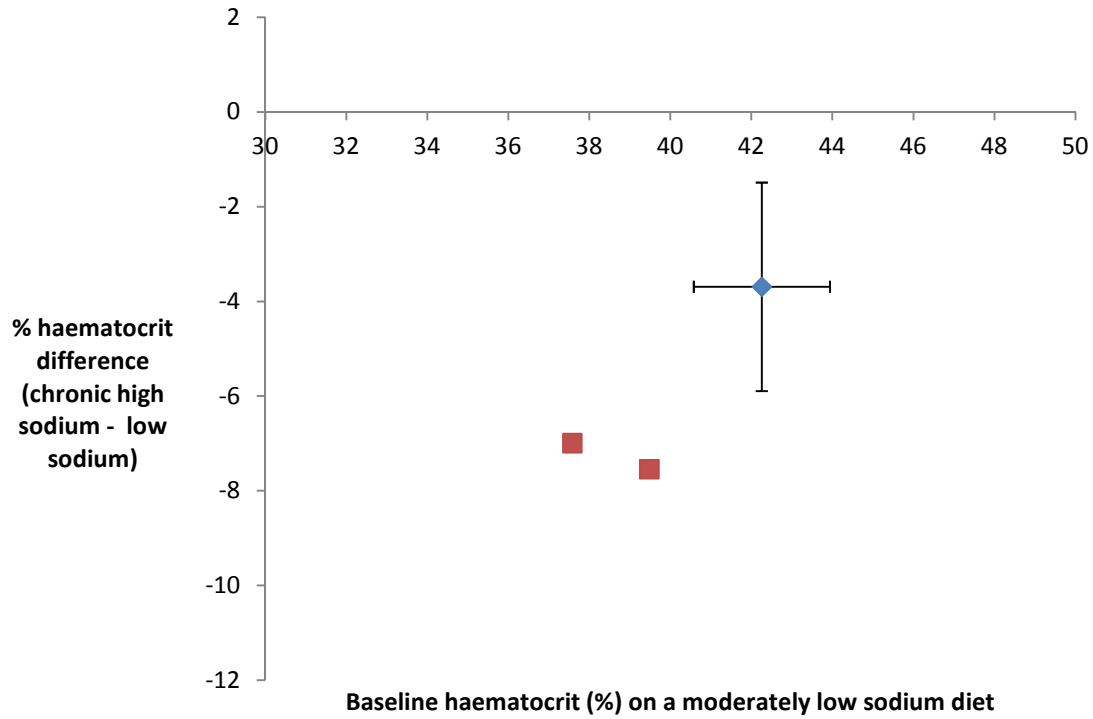


Figure 2.20 – Percentage difference in haematocrit between exposure to a moderately low sodium diet and a chronic high sodium load for thiazolidinedione (TZD) tolerant [n = 8 (low sodium), n = 9 (chronic high sodium), plotted in blue] and intolerant (n = 2, plotted in red) subjects. Mean and 95% confidence intervals were derived for TZD tolerant subjects.



2.22 Weight change in response to salt loading

All participating patients tended to gain weight on progressing from a low to a high salt diet. Exploratory data suggest no significant differences between thiazolidinedione categories (table 2.20). Plotting % change in weight secondary to dietary adjustments yielded a mean (95% CI) increase of 0.67 (0.20, 1.14)% for thiazolidinedione tolerant patients. TZD intolerant patient 11 exhibited a substantially greater increase in body weight on chronic sodium exposure (2.14%); however, this result was not replicated in intolerant subject 10 (table 2.20).

Table 2.20 - Body weight (kg) and derived % differences between sodium load exposures for visits 2 and 3.

Subject number by category	*Body weight (low sodium)* *(kg)*	*Body weight (chronic high sodium)* *(kg)*	*% difference body weight (acute high sodium - low sodium)*
TZD tolerant			
1	74.8	76.4	2.14
2	62.8	63.3	0.80
3	92.1	92.6	0.54
4	89.2	89.0	-0.22
5	88.4	89.4	1.13
6	98	98.6	0.61
7	93.6	93.4	-0.21
8	118.6	119.0	0.34
9	86.0	86.8	0.93
Mean	**89.3**	**89.8**	**0.67**
(95% CI)	**(79.25, 99.31)**	**(79.91, 99.75)**	**(0.20, 1.14)**
TZD intolerant			
10	88.6	88.8	0.23
11	84.0	85.8	2.14

2.23 Ankle-foot volume changes in response to dietary sodium exposure

Data from participating thiazolidinedione tolerant and intolerant subjects are summarised in table 2.21, figures 2.21 to 2.23 below. One thiazolidinedione tolerant subject declined to pursue with AFV measurements after exposure to intravenous 0.9% saline infusion (acute sodium load); hence AFV data for acute sodium load exposure are limited to eight subjects. Mean (95% CI) for the %AFV difference between acute high sodium load exposure and low dietary sodium exposure for thiazolidinedione tolerant subjects amounted to 2.5 (-2.2, 7.2)%. The corresponding values for the difference between chronic high and low dietary sodium exposure was 2.2 (0.3, 4.1)%. Available data suggest that exposure to a acute high sodium load may result in a reduction in AFV in thiazolidinedione intolerant subjects, but not in TZD tolerant patients (table 2.21, figure 2.22). However, data must be interpreted

with caution, given (i) the small number of participating subjects in each thiazolidinedione category, and (ii) wide 95% CIs for AFV change in thiazolidinedione 'tolerant' subjects. TZD intolerant subject 11 reduced her AFV by a greater extent than her 'tolerant counterparts in response to acute salt loading [-2.4% vs mean (95% CI) 2.5 (-2.2, 7.2)% for 'tolerant subjects]. A similar, thought seemingly insignificant change, was reported for intolerant subject 10 (table 2.21, figure 2.22). Analyzing percentage change in AFV following five days of high sodium intake, TZD intolerant subject 11 was characterised by a greater increase [5.5% vs mean (95% CI) 2.2 (0.3, 4.1)% for thiazolidinedione tolerant subjects]. TZD intolerant subject 10's % AFV increase was similar to that of TZD tolerant patients subjected to chronic sodium loading (table 2.21, figure 2.23).

Table 2.21 - Ankle-foot volume (AFV) measurements and derived % differences between sodium load exposures for visits 2 and 3

Subject number by category	*AFV (low Na)*	*AFV (acute high Na)*	*AFV (chronic high Na)*	*% difference AFV (acute high Na - low sodium)*	*% difference AFV (chronic high Na - low sodium)*
TZD tolerant					
1	2779	[a]	3013	[b]	8.4
2	2394	2868	2497	19.8	4.3
3	3101	3085	3100	-0.5	0.0
4	3167	3254	3194	2.7	0.9
5	2875	2872	2858	-0.1	-0.6
6	3617	3654	3630	1.0	0.4
7	3055	3013	3106	-1.4	1.7
8	3559	3613	3706	1.5	4.1
9	3548	3441	3569	-3.0	0.6
Mean (95% CI)	**3122 (2855, 3389)**	**3225 (3006, 3444)**	**3186 (2929, 3443)**	**2.5 (-2.2, 7.2)**	**2.2 (0.3, 4.1)**
TZD intolerant					
10	3572	3503	3627	-1.9	1.5
11	2743	2677	2894	-2.4	5.5

[a] Patient declined to measure ankle-foot volume by ankle displacement on this occasion; [b] calculation not possible due to missing data.

Figure 2.21 – Mean (95% CI) ankle-foot volume (AFV) readings (mLs) for thiazolidinedione (TZD) tolerant [n = 9 (low sodium diet); n = 8 (acute high sodium load), plotted in blue) and individual AFV readings for thiazolidinedione intolerant (n = 2, plotted in red) subjects exposed to a low sodium diet, an acute high sodium load and a chronic high sodium load.

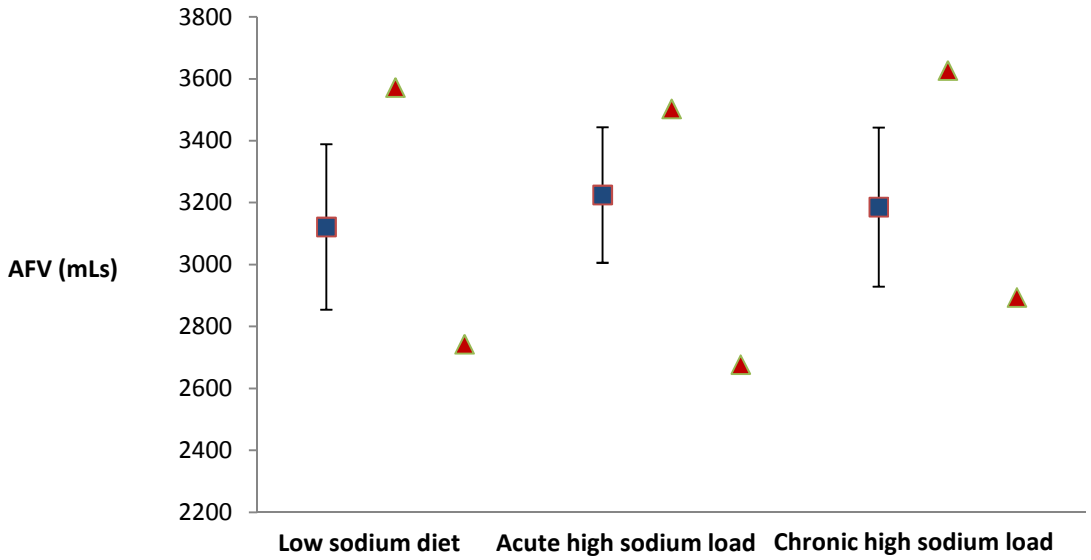


Figure 2.22 – Percentage difference in ankle-foot volume (AFV) between exposure to a moderately low sodium diet and an acute high sodium load for thiazolidinedione (TZD) tolerant [n = 9 (low sodium diet); n = 8 (acute high sodium load) plotted in blue) and intolerant (n = 2, plotted in red) subjects. Mean and 95% confidence intervals were derived for TZD tolerant subjects.

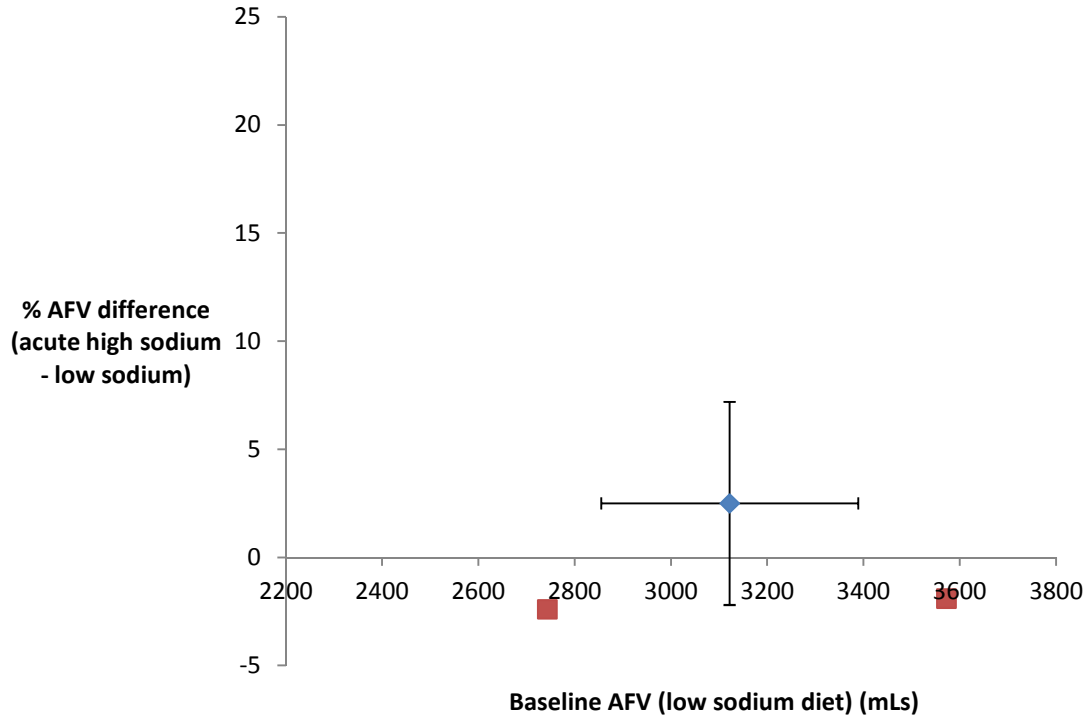
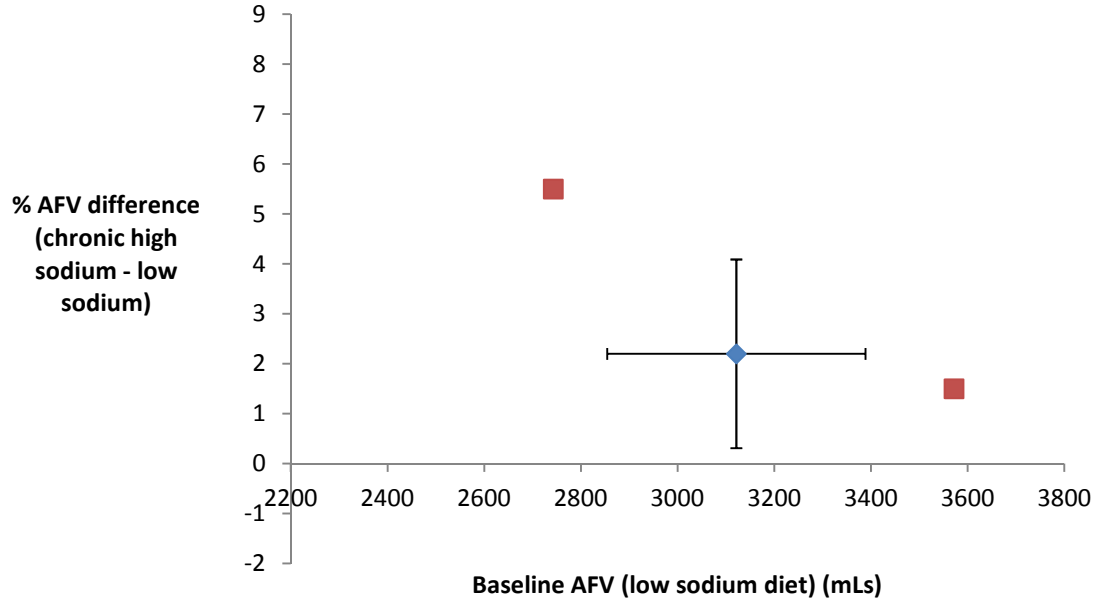


Figure 2.23 – Percentage difference in ankle-foot volume (AFV) between exposure to a moderately low sodium diet and a chronic high sodium load for thiazolidinedione (TZD) tolerant (n = 9, plotted in blue) and intolerant (n = 2, plotted in red) subjects. Mean and 95% confidence intervals were derived for TZD tolerant subjects.



2.24 Salt sensitivity of blood pressure

SBP and DBP values were compared at the beginning of visits 2 and 3, enabling an assessment of salt sensitivity in response to a chronic salt loading. Mean arterial pressure (MAP) values were derived for each patient at each time-point using the formula [(2*DBP) + SBP]/3.

2.24.1 Systolic blood pressure

Thiazolidinedione tolerant patients exhibited no significant shift in their baseline SBP readings when progressing from a moderately low sodium diet to a chronic high sodium diet [mean (95% CI) SBP = 138.6 (132.3, 144.9) (low sodium) vs 138.1 (130.7, 145.5) (chronic high sodium)]. The two participating 'intolerant' subjects

shifted their SBP readings in either direction, as outlined in appendix table x.x Derived percentage SBP shifts across sodium load categories were more marked for either thiazolidinedione intolerant subject, albeit in opposite directions, as outlined in appendix table II.8.

2.24.2 Diastolic blood pressure

Thiazolidinedione tolerant patients' DBP readings showed only marginal change on progressing from a moderately low sodium diet [mean (95% CI) DBP = 83.4 (79.0, 87.8) mmHg) to a chronic high sodium diet [mean (95% CI) DBP = 83.1 (78.3, 87.9) mmHg], as outlined in table 2.22 and figure 2.24. Exploratory data suggest that mean (95% CI) percentage DBP change for thiazolidinedione tolerant subjects [-0.2 (-4.1, 3.7)] is lower than the individual % DBP reduction values for intolerant patients (-7.5%, -4.6% respectively) (table 2.22, figure 2.25).

Table 2.22 - Diastolic blood pressure (DBP) readings (mmHg) and derived % differences between sodium load exposures for visits 2 and 3.

Subject number by category	*DBP (mmHg) (low sodium)*	*DBP (mmHg) (chronic high sodium)*	*% difference DBP (chronic high sodium - low sodium)*
TZD tolerant			
1	84.3	89.7	6.3
2	74.0	73.0	-1.4
3	76.7	84.0	9.6
4	91.0	92.0	1.1
5	75.7	70.7	-6.6
6	88.0	87.0	-1.1
7	87.3	80.0	-8.4
8	92.0	88.0	-4.3
9	81.7	84.0	2.9
Mean	**83.4**	**83.1**	**-0.2**
(95% CI)	**(79.0, 87.8)**	**(78.3, 87.9)**	**(-4.1, 3.7)**
TZD intolerant			
10	93.3	86.3	-7.5
11	93.7	89.3	-4.6

Figure 2.24 – Mean (95% CI) diastolic blood pressure (DBP) values (mmHg) for thiazolidinedione (TZD) tolerant (n = 9, plotted in blue) and individual systolic blood pressure readings for thiazolidinedione intolerant (n = 2, plotted in red) subjects exposed to a low sodium diet and a chronic high sodium load.

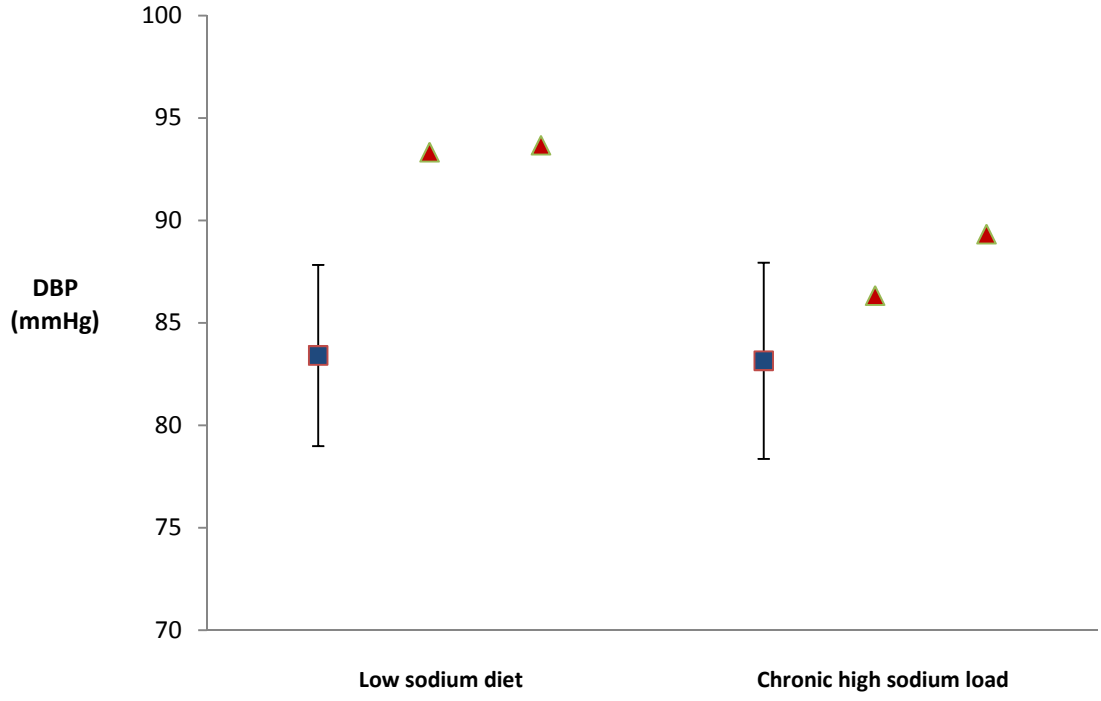
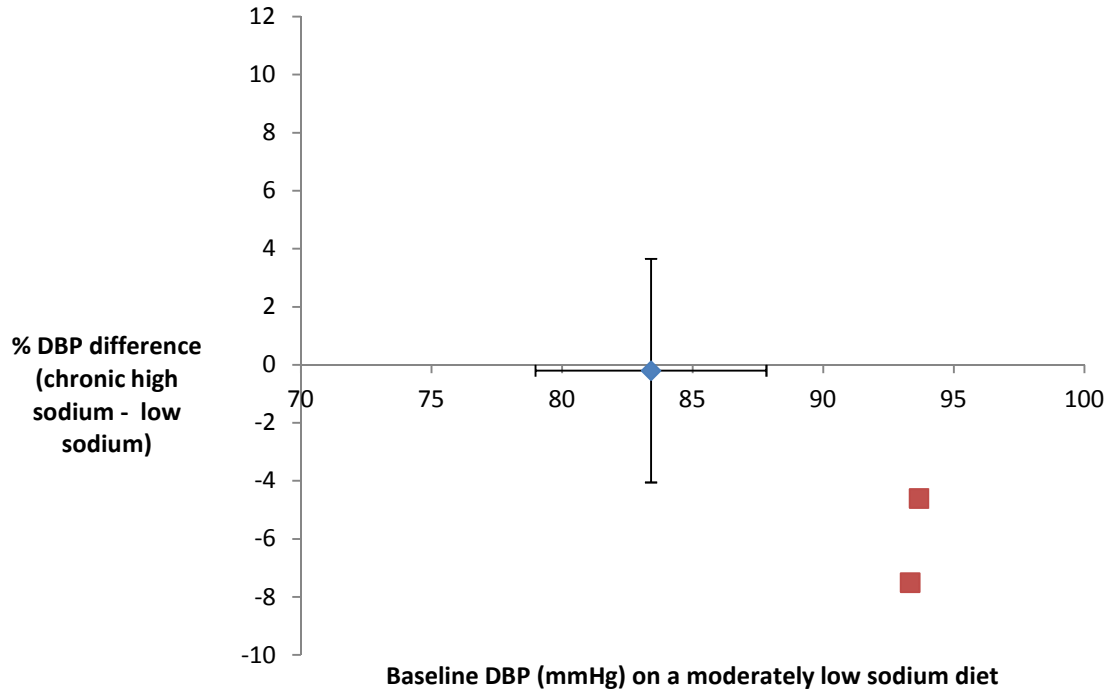


Figure 2.25 – Percentage difference in diastolic blood pressure (DBP) readings (mmHg) between exposure to a moderately low sodium diet and exposure to a chronic high sodium load for thiazolidinedione (TZD) tolerant (n = 9, plotted in blue) and intolerant (n = 2, plotted in red) subjects. Mean and 95% confidence intervals were derived for thiazolidinedione tolerant subjects.



2.24.3 Mean arterial pressure

Comparing MAP values following exposure to five days of a moderately low sodium and another five days of chronic salt loading, thiazolidinedione 'tolerant' subjects were characterised by a marginal change in their prevalent MAP [mean (95% CI) MAP = 101.8 (98.2, 105.4) mmHg (low sodium diet) vs 101.5 (97.1, 105.8) (high sodium diet)] mm Hg (appendix table II.9). While thiazolidinedione intolerant subject 11 exhibited a 2.9 mmHg increase in MAP in response to chronic salt loading (2.6% increase over baseline MAP), almost rendering her salt sensitive, intolerant subject 10 exhibited a 12.3 mmHg (10.7%) shift in the opposite direction, rendering comparisons across thiazolidinedione categories equivocal (appendix table II.9).

2.25 Deuterium analysis

Total body water (TBW), measured in kg, and percentage total body water (% TBW, relative to total body mass) were determined using deuterium analysis, as discussed earlier. FFM, FM, percentage FFM and percentage FM were derived from individual patients' TBW values, as outlined in section I.

2.25.1 Total body water estimation

Mean (95% CI) derived TBW and % TBW readings for thiazolidinedione tolerant patients were 39.76 (34.59, 44.93) kg and 44.42 (40.42, 48.42)% respectively. Available data (table 2.23) suggest no difference in TBW or % TBW between thiazolidinedione tolerant or intolerant patients.

Table 2.23 - Total body water (TBW) measurements (kg) and derived % TBW values for thiazolidinedione 'tolerant' and 'intolerant' patients exposed to a moderately high sodium diet.

Subject number by category	*Weight (kg)*	*Height (cm)*	*Body mass index (kg/m^2)*	*Mean (SD) true TBW (kg)[a]*	*% TBW*
TZD tolerant					
1	76.4	168	27.07	33.88 (0.78)	44.35
2	63.3	160	24.73	30.23 (0.59)	47.76
3	92.6	180	28.58	52.94 (0.33)	57.17
4	89.0	165	32.69	42.27 (0.41)	47.50
5	89.4	160	34.92	33.16 (0.92)	37.09
6	98.6	172	33.33	42.68 (0.18)	43.29
7	93.4	174	30.85	41.34 (1.03)	44.26
8	119.0	180	36.73	48.99 (0.12)	41.17
9	86.8	160	33.91	32.30 (0.02)	37.21
Mean (95% CI)	**89.8 (79.91,99.75)**	**169 (163.6,174.4)**	**31.42 (28.85, 33.99)**	**39.76 (34.59, 44.93)**	**44.42 (40.42, 48.42)**
TZD intolerant					
10	88.8	157	36.03	38.06 (1.06)	42.86
11	85.8	160	33.52	31.88 (0.20)	37.16

2.25.2 Fat-free mass and fat mass

Mean (95% CI) FFM values for thiazolidinedione tolerant patients was 54.31 (47.25, 61.37) kg while the corresponding % FFM amounted to 60.69 (55.22, 66.16) % (table 2.24). TZD tolerant patients were characterised by a mean (95% CI) FM of 35.52 (28.65, 42.39) and a mean % FM of 39.31 (33.84, 44.78) (table 2.24). Available data suggest no significant differences in FM, FFM, % FM or % FFM between either thiazolidinedione subgroup. Fat-free mass index (FFMI) and fat mass index (FMI) were derived by dividing each FFM and FM value by body weight in kg and expressed as kg/m^2. Mean (95% CI) FFMI and FMI for thiazolidinedione 'tolerant' patients amounted to 18.89 (17.45, 20.33) and 12.53 (10.08, 14.98) respectively (table 2.24). Exploratory data suggest that TZD intolerant may be characterised by a higher FMI than their oedema free counterparts (95% CI for the latter treatment group only marginally overlap individual data points for the former). However, there seems be no difference in FFMI values between either thiazolidinedione cohort, with individual plots for TZD intolerant patients being on either side of the 95% CI range for 'tolerant' subjects (table 2.24).

Table 2.24 - Derived fat-free mass (FFM) and fat mass (FM) measurements (kg), and derived % FFM and % FM for thiazolidinedione 'tolerant' and 'intolerant' patients exposed to a moderately high sodium diet).

Subject number by category	*Fat free mass (kg)*	*% fat free mass*	*Fat mass (kg)*	*% fat mass*	*Fat free mass index (kg/m^2)*	*Fat mass index (kg/m^2)*
TZD tolerant						
1	46.29	60.58	30.11	39.42	16.40	10.67
2	41.30	65.25	22.00	34.75	16.13	8.59
3	72.32	78.10	20.28	21.90	22.32	6.26
4	57.75	64.88	31.25	35.12	21.21	11.48
5	45.30	50.67	44.10	49.33	17.70	17.23
6	58.31	59.14	40.29	40.86	19.71	13.62
7	56.48	60.47	36.92	39.53	18.65	12.20
8	66.93	56.24	52.07	43.76	20.66	16.07
9	44.12	50.83	42.68	49.17	17.23	16.67
Mean (95% CI)	**54.31 (47.25, 61.37)**	**60.69 (55.22, 66.16)**	**35.52 (28.65, 42.39)**	**39.31 (33.84, 44.78)**	**18.89 (17.45, 20.33)**	**12.53 (10.08, 14.98)**
TZD intolerant						
10	51.99	58.55	36.81	41.45	21.09	14.93
11	43.55	50.76	42.25	49.24	17.12	16.61

2.26 Inulin clearance

Glomerular filtration rate was measured using the inulin clearance (InCl) method at each of the three salt loading states, as outlined in section I. Mean (95% CI) InCl readings for TZD tolerant patients increased following both acute and chronic salt exposure - with the magnitude of change being higher and likely significant in response to the former [13.78 (8.33, 19.23)% (acute high sodium exposure) vs 4.39 % (-0.04, 8.82) % (chronic high sodium exposure)] (table 2.25). Individual readings for thiazolidinedione intolerant subject 10 (84.58 ml/min) exceeded the upper end of the 95% CI range for tolerant patients after intravenous saline infusion [mean (95% CI) = 68.95 (63.93, 73.97) ml/min]. However, this result was not replicated in TZD

intolerant subject 11. In contrast, the latter's InCl value marginally exceeded the mean (95% CI) readings for tolerant subjects exposed to a five day high salt diet [69.60 (subject 11) vs 64.54 (59.69, 69.39) (thiazolidinedione tolerant) mL/min] (table 2.25). Intolerant subject 10's percentage increase in glomerular filtration rate in response to acute high salt loading exceeded that for TZD tolerant patients [33.90% (subject 10) vs 13.78 (8.33, 19.23)% (thiazolidinedione tolerant)]. A similar pattern of difference in percentage change was only replicated in intolerant subject 11 following chronic salt loading [18.8% (subject 11) vs 4.39 (-0.04, 8.82)% (thiazolidinedione tolerant)] (table 2.25).

Table 2.25 - Inulin clearance (InCl) and derived % differences between sodium load exposures for visits 2 and 3.

Subject number by category	*InCl (low Na) (mL/min)*	*InCl (acute high Na) (mL/min)*	*InCl (chronic high Na) (mL/min)*	*% difference InCl (acute high Na - low Na)*	*% difference InCl (chronic high Na - low Na)*
TZD tolerant					
1	66.27	[a]	75.24	[b]	13.53
2	61.68	67.07	68.79	8.75	11.53
3	68.09	79.67	69.02	17.00	1.37
4	54.38	69.62	51.50	28.02	-5.30
5	61.47	64.51	66.08	4.94	7.49
6	61.71	70.15	60.73	13.68	-1.59
7	64.94	73.45	66.89	13.10	2.99
8	52.46	58.21	55.15	10.97	5.13
9	[a]	[a]	67.46	[a]	[a]
Mean (95% CI)	**61.38 (57.58, 65.18)**	**68.95 (63.93, 73.97)**	**64.54 (59.69, 69.39)**	**13.78 (8.33, 19.23)**	**4.39 (-0.04, 8.82)**
TZD intolerant					
10	63.16	84.58	65.47	33.90	3.65
11	58.58	69.39	69.60	18.44	18.80

[a] Data unavailable; [b] calculation not possible due to missing data

2.27 Fractional excretion of sodium

FeNa values were derived for nine, six and seven thiazolidinedione tolerant subjects at low sodium, acute high sodium and chronic sodium loading states respectively, and from both intolerant patients at all stages of sodium exposure (table 2.26). As expected, most thiazolidinedione-treated subjects increased their FeNa in response to chronic salt loading. Percentage change in FeNa was more marked in response to a five day high sodium diet compared with acute salt loading for either thiazolidinedione subgroup (table 2.26). Mean (95% CI) FeNa values for thiazolidinedione tolerant subjects overlapped across all three sodium load categories. Individual plots for oedema prone patient 11 suggested significantly lower FeNa values when exposed to a low sodium diet and acute salt loading (table 2.26). This individual was also characterised by a particularly marked percentage increase in her FeNa on chronic salt loading [in excess of 4.5 fold increase over baseline low sodium FeNa reading vs mean (95% CI) value of 115.14 (11.48, 218.80)% for thiazolidinedione tolerant subjects]. However, these results were not replicated in intolerant subject 10.

Table 2.26 - Fractional excretion of sodium (FeNa) and derived % differences between sodium load exposures for visits 2 and 3.

Subject number by category	*FeNa (low Na) (%)*	*FeNa (acute high Na) (%)*	*FeNa (chronic high Na) (%)*	*% difference FeNa (acute high Na - low Na)*	*% difference FeNa (chronic high Na - low Na)*
TZD tolerant					
1	0.98	[a]	[a]	[b]	[b]
2	0.56	[a]	[a]	[b]	[b]
3	0.44	0.70	0.99	59.16	127.12
4	1.08	0.86	1.45	-20.35	33.84
5	0.09	0.18	0.45	109.81	416.11
6	0.37	0.23	0.63	-36.42	71.30
7	0.29	0.41	0.45	42.89	54.51
8	0.21	0.32	0.43	55.32	108.40
9	0.95	[a]	0.90	[a]	-5.32
Mean (95% CI)	**0.55 (0.31, 0.79)**	**0.45 (0.23, 0.67)**	**0.76 (0.48, 1.04)**	**35.07 (-8.49, 78.63)**	**115.14 (11.48, 218.80)**
TZD intolerant					
10	0.56	0.68	1.14	21.57	103.17
11	0.15	0.20	0.85	33.72	455.63

[a] Data unavailable; [b] calculation not possible due to missing data.

2.28 Fractional excretion of lithium

FeLi data could be accrued from eight, six and seven thiazolidinedione tolerant patients exposed to a moderately low sodium diet, acute saline infusion and chronic salt loading respectively, and from two intolerant patients for each of the three salt loading states (table 2.27). Exploratory data suggest that TZD intolerant subject 10 was characterised by a significantly higher FeLi than tolerant patients in response to acute and chronic salt loading. These results were partially replicated in intolerant subject 11, with the latter exhibiting a higher FeLi following exposure to a high salt diet (17.46%), albeit marginally lower FeLi (7.57%) under low salt conditions (table 2.27). Analyzing for percentage change in FeLi across sodium categories,

thiazolidinedinone intolerant subject 11 was characterised by a greater increase over baseline (low sodium diet) on both acute and chronic salt loading. TZD intolerant subject 10's % FeLi change was within the 95% CI range for 'tolerant' subjects after exposure to one litre of intravenous saline, and exhibited a reduction in FeLi in response to chronic salt loading (table 2.27).

Table 2.27 - Fractional excretion of lithium (FeLi) and derived % differences between sodium load exposures for visits 2 and 3.

Subject number by category	*FeLi (low Na) (%)*	*FeLi (acute high Na) (%)*	*FeLi (chronic high Na) (%)*	*% difference FeLi (acute high Na - low Na)*	*% difference FeLi (chronic high Na - low Na)*
TZD tolerant					
1	18.09	[a]	[a]	[b]	[b]
2	40.00	[a]	[a]	[b]	[b]
3	19.28	15.43	15.17	-19.99	-21.34
4	14.93	13.77	17.53	-7.77	17.42
5	7.51	8.15	11.26	8.51	50.04
6	10.83	9.76	11.63	-9.96	7.36
7	7.40	12.19	11.16	64.82	50.90
8	10.51	11.07	10.80	5.29	2.74
9	[a]	[a]	18.35	[b]	[b]
Mean (95% CI)	**16.07 (8.68, 23.46)**	**11.73 (9.6, 13.86)**	**13.7 (11.29, 16.11)**	**6.82 (-17.40,31.04)**	**17.85 (-4.79, 40.49)**
TZD intolerant					
10	17.67	19.63	16.33	11.10	-7.56
11	7.57	11.96	17.46	58.04	130.71

[a] Data unavailable; [b] calculation not possible due to missing data.

2.29 Fractional reabsorption of distally delivered sodium

Fractional reabsorption of distally delivered sodium (FRDDNa) was calculated for each participant from FeNa and FeLi values, as outlined in section I. Data were accrued from eight, six and seven thiazolidinedione tolerant subjects after low, acute

high and chronic high sodium exposure respectively, and from each of the two intolerant subjects at each instance.

Table 2.28 - Fractional reabsorption of distally delivered sodium (FRDDNa) and derived % differences between sodium load exposures for visits 2 and 3.

Subject number by category	*FRDDNa (low Na) (%)*	*FRDDNa (acute high Na) (%)*	*FRDDNa (chronic high Na) (%)*	*% difference FRDDNa (acute high Na - low Na)*	*% difference FRDDNa (chronic high Na - low Na)*
TZD tolerant					
1	94.58	[a]	[a]	[b]	[b]
2	98.60	[a]	[a]	[b]	[b]
3	97.72	95.46	93.47	-2.31	-4.34
4	92.77	93.75	91.73	1.07	-1.12
5	98.80	97.79	96.00	-1.02	-2.83
6	96.58	97.64	94.58	1.10	-2.07
7	96.08	96.64	95.97	0.58	-0.12
8	98.00	97.11	96.02	-0.91	-2.02
9	[a]	[a]	95.10	[b]	[b]
Mean (95% CI)	**96.64 (95.18, 98.10)**	**96.40 (95.17, 97.63)**	**94.70 (93.51, 95.89)**	**-0.25 (-1.35, 0.85)**	**-2.08 (-3.24, -0.92)**
TZD intolerant					
10	96.83	96.54	93.02	-0.30	-3.94
11	98.02	98.33	95.13	0.32	-2.95

[a] Data unavailable; [b] calculation not possible due to missing data

Comparing with mean (95% CI) values for thiazolidinedione tolerant patients, TZD intolerant subject 11 (albeit not subject 10) was characterised by a higher absolute FRDDNa value in response to acute salt loading. TZD intolerant subject 10 exhibited a lower FRDDNa than her tolerant counterparts in response to a five day moderately high sodium diet (table 2.28). No differences in % shifts in FRDDNa between thiazolidinedione categories were reported in response to acute salt loading. Thiazolidinedione intolerant subject 11 decreased her FRDDNa to a greater extent

than her tolerant counterparts in response to chronic salt loading; however, this result was not replicated in TZD intolerant subject 11 (table 2.28).

2.30 Discussion

This study sought to investigate the hypotheses that patients previously known to be intolerant to thiazolidinediones would be characterised during either acute or chronic 'high normal' sodium loading by impaired left ventricular diastolic function, high pulse wave velocity and higher plasma VEGF levels. There are two main theories of the mechanisms underpinning the development of oedema. Secondary (underfill) oedema results from a renal response to actual or sensed underfilling of the effective arterial blood volume (EABV). The resulting reduction in tissue perfusion sets forth a physiologically appropriate retention of sodium and water by the kidneys through activation of the sympathetic nervous system, the renin-angiotensin-aldosterone system and vasopressin release, and an increase in circulating catecholamines [540]. The relative contribution of VEGF in the aetiopathogenesis of thiazolidinedione-associated oedema remains controversial. This study did not observe differences in VEGF according to tolerance of thiazolidinediones; moreover, no differences in any of the measured echocardiographic indices were detected between TZD tolerant and intolerant patients.

Available evidence suggests that thiazolidinedione therapy may be primarily associated with inappropriate renal sodium handling. Renal PPAR-γ receptors are primarily concentrated in the collecting tubules [287], a major site of sodium and water retention occurring primarily under aldosterone, and to a lesser extent AVP,

ANP and insulin control [541]. PPAR-γ-mediated ENaC activity may favour sodium reabsorption and an increase in extracellular fluid volume [302, 303], causing an expansion of its subcompartments, manifesting clinically as oedema. In accordance with the overfill theory, this would be expected to enhance the normal central inhibition of the sympathetic nervous system, while suppressing both the renin-angiotensin-aldosterone pathway and baroreceptor mediated AVP release [540].

Thiazolidinedione intolerant patients exhibited a greater decrease in haematocrit, and increase in cAI and pAI (surrogate markers of arterial stiffness) in response to salt loading. This would suggest a preservation of the EABV. Thiazolidinedione therapy has been associated with a reduction in aldosterone [303, 304, 541] and an increase in ANP [335, 336] in published studies of animal models and human subjects. ANP is synthesised and stored (within granules) as pre-pro-ANP, cleaved to pro-ANP_{1-126}, and secreted as the biologically active 28 amino acid peptide (together with biologically inactive N-terminal pro-ANP) [542]. These physiological processes proceed in the atria in response to increased atrial pressure and distension [543], occurring as a result of acute volume expansion, salt feeding, water immersion and postural changes. BNP is likewise synthesised as a 134 amino acid peptide called pre-pro-BNP, cleaved into pro-BNP_{1-108} [544, 545], and secreted in bursts as biologically active BNP_{1-32} (together with its inactive amino-terminal fragment NT-pro-BNP) [545-547]. This synthetic activity progresses primarily in the ventricular myocardium, particularly in response to volume expansion [548, 549]. Both pro-ANP and pro-BNP are cleaved into biologically active ANP and BNP by the cardiac myocyte transmembrane enzyme corin, a member of the serine protease family [550]. ANP and BNP play a pivotal role in salt and water homeostasis by increasing

glomerular filtration and filtration fraction despite a fall in mean arterial pressure [551]. Moreover they decrease sodium reabsorption in the cortical collecting tubule and inner medullary collecting duct (independently of effects on glomerular filtration) [552], decrease passive sodium chloride reabsorption in the thin ascending limb [553], reduce renin secretion, block aldosterone secretion and oppose the vasoconstrictive effect of angiotensin II [549, 554, 555]. This study did not report differences in aldosterone, renin, ANP and BNP concentrations between thiazolidinedione tolerant patients and those whose thiazolidinedione therapy was complicated by oedema and /or HF. However, TZD intolerant patients were characterised by a significantly greater reduction in their circulating renin (and possibly aldosterone), and a greater increase in ANP levels (and possibly BNP) following chronic salt loading. These observations are consistent with the overfill theory. Copeptin is a 39 amino-acid peptide released together with AVP during processing of the precursor peptide pro-AVP, and has proven to be a useful surrogate marker of circulating AVP [556]. AVP secretion is favoured by hypovolaemia, increased serum osmolality [556, 557], angiotensin II and norephinephrine [558]. As expected, high sensitivity copeptin decreased in response to chronic salt loading in thiazolidinedione tolerant subjects. Physiologically, this would be an expected response in the face of an increased EABV (as attested by an accompanying fall in haematocrit). 'Oedema-prone' subject 10's copeptin level was lower than the mean (95% CI) range for 'tolerant' subjects, in keeping with the overfill theory. Perhaps surprisingly, 'intolerant' subject 11 increased her copeptin on chronic salt loading. This study's small dataset precludes from judging whether this is a stress response [556] or a result worthy of further investigation.

In their study on healthy, normotensive male volunteers subjected to both low and high salt diets, Zanchi et al. reported that thiazolidinedione therapy is associated with a significant increase in plasma renin activity in both instances, as well a rise in daytime heart rate, which however, only reached statistical significance in low salt loading states. In the absence of a significant effect on nocturnal (supine) blood pressure, the authors ascribed this to thiazolidinedione-associated peripheral vasodilation [322]. Results arising from this study seem to imply that 'oedema-prone' patients are less likely to peripherally vasodilate in response to chronic salt loading, further enhancing intravascular volume, increasing atrial stretch (and hence ANP and BNP release), while propogating a further inhibition of the renin-angiotensin-aldosterone pathway. While thiazolidinedione-mediated increases in ANP should theoretically mitigate any drug-associated oedema, this effect is blunted in T2DM patients [335].

Although the synthetic activity of atrial and cardiac myocytes may be overwhelmed in severe HF (creating a relative deficiency) [559, 560], resistance to the effects of natriuretic peptides has been suggested as a possible contributory mechanism in the aetiopathogenesis of fluid overload. ANP and BNP bind to the natriuretic peptide A receptor (NPR-A) and exert their hormonal effects via 3', 5' - cyclic guanosine monophosphate (cGMP). Both natriuretic peptides are cleared via the natriuretic peptide C receptor (NPR-C), and degraded by the ectoenzyme neutral endopeptidase 24.11 (NEP). ANP and/or BNP resistance reportedly arise as a consequence of decreased corin activity, down-regulation of NPR-A, increased metabolism of cGMP by cGMP phosphodiesterase V or increased clearance of the natriuretic peptides by NPR-C or NEP [561-564]. A higher relative increase in ANP and BNP levels in

response to chronic salt loading among thiazolidinedione 'intolerant' patients in this exploratory study could thus be ascribed to natriuretic peptide resistance.

There is currently considerable interest in the association of common genetic variants at the Natriuretic Peptide Precursor A (NPPA) - Natriuretic Peptide Precursor B (NPPB) locus on chromosome 1 with circulating ANP and BNP concentrations. Cheh et al. reported that genetic variants rs5068 and rs198358 are associated with higher ANP concentrations, lower SBP and DBP values, and a lower risk of hypertension in a 14,743 individuals of European ancestry with no prior HF participating in the Framingham study [565]. These effects are not entirely surprising given ANP's modulation of natriuresis and vascular tone. Cannone et al. additionally associated genetic variant rs5068 with a better cardiometabolic profile (lower BMI, lower prevalence of obesity, lower waist circumference, lower C-reactive protein, higher high-density lipoprotein cholesterol), albeit no association with an altered risk for hypertension, congestive HF, coronary artery disease, atrial fibrillation or cerebrovascular accident in a study of 1608 randomly selected US subjects [566]. Similar results were reported in a Mediterranean population [567]. Moreover, the corin I555 (P568) allele, particularly common in blacks, has been associated with higher blood pressure and a higher prevalence of hypertension in a genotype-phenotype genetic association study of US patients [568]. This study described considerable differences in prevalent ANP levels between individual subjects participating in this small exploratory study, particularly among those pertaining to the thiazolidinedione tolerant subgroup. While it would be unwise to draw specific conclusions, such differences could well be ascribed to genetic variants, and may be worthy of further study.

This study also sought to investigate the hypotheses that patients prone to thiazolidinedione-associated oedema and HF are characterised by increased AFV, salt sensitivity of blood pressure and differences in fractional sodium excretion, free water handling and total body water when compared with thiazolidinedione tolerant subjects. A decline in haematocrit and an accompanying increase in total body weight was reported in response to chronic salt loading for all subjects. This observation would be consistent with a tendency to fluid overload. The degree of haemodilution following acute compared with chronic salt loading tended to be greater within thiazolidinedione tolerant subjects suggesting a role for compensatory physiological mechanisms which become more effective in the 'longer' rather than 'shorter' term. In contrast, patients whose thiazolidinedione therapy was previously complicated by oedema were characterised by a greater degree of haemodilution following chronic salt loading. This could imply a relative failure of counter-regulatory mechanisms.

Thiazolidinedione intolerant subject 11 virtually fulfils the criterion for salt-sensitivity of blood pressure (SSBP) (being characterised by a 2.9 mmHg increase in MAP following chronic salt loading). This patient was also characterised by a 17.3 mmHg increase in SBP following exposure to a chronic high salt diet. Oedema prone subject 10 shifted her MAP and SBP in the opposite direction. This study's reported mean (95% CI) reductions in SBP and MAP for thiazolidinedione 'tolerant' patients are perhaps surprising, and generally contrast with those reported in the literature. The INTERSALT study analyzed data from 10,079 patients aged 20 to 59 recruited from 52 centres across 32 countries. Multivariate analysis with and without BMI in the analysis showed that a 100 mmol daily dietary salt reduction results in a 3.1-6.0

mmHg and a 0.1 -6.0 mmHg reduction in SBP and DBP respectively [569]. The Dietary Approach to Stop Hypertension (DASH) study published data on the effect of the DASH diet and dietary salt restriction (<100 mmol/day) on SBP, claiming it is associated with a 7.1 mmHg reduction in normotensive individuals and a 11.5 mmHg reduction in hypertensive individuals compared to controls exposed to a high salt diet [570]. A recent Cochrane systematic review and meta-analysis of 34 trials recruiting 3230 participants concluded that a 75 mmol reduction in daily urinary sodium excretion (equivalent to a reduction of 4.4 g/day) for at least four weeks is associated with a 4.18 mmHg reduction in prevalent SBP (95% CI - 5.18, -3.18) and a 2.06 mmHg reduction in DBP (95% CI -2.67, -1.45), and that this was associated with a small physiological increase in plasma renin activity, aldosterone and noradrenaline (albeit no significant change in lipid concentrations) [571]. In a study of 70 Hong Kong Chinese patients with untreated hypertension and 47 normotensive controls, DBP correlated with 24 hour urinary sodium excretion in hypertensive patients, but not in controls [572]. Despite their undisputed validity, these studies findings' may not be directly relevant to this exploratory study, particularly as they either (i) recruited patients whose dietary sodium intake was modified for substantially longer periods (>30 days) [570, 571] or (ii) observed the effects of long-term (usual) dietary sodium habits [569, 572]. Closer to this study's design, Foo et al. analysed the impact of a 6-day high (220 mmol/day) and low (40 mmol/day) sodium diet on blood pressure, leg flow and insulin sensitivity in 18 healthy normotensive subjects. Salt loading was associated with a borderline significant increase in 24-hour SBP [mean (SD) = +5.8 (± 14.2) mmHg], but no significant impact on DBP or MAP [573]. Twenty healthy normotensive volunteers were recruited into another study investigating the impact of dietary salt on insulin sensitivity. Although Townsend et

al. reported a 6/4 mmHg BP increase on progressing from a six-day 20 mmol/day sodium diet to a six-day 200 mmol/day diet, this change did not reach statistical significance [574]. Vedovato et al. examined the impact of a seven-day low (20 mmol) and seven-day high (250 mmol) sodium diet on MAP and other parameters in a cohort of 20 T2DM patients with microalbuminuria and 21 T2DM patients without microalbuminuria. 24-hour MAP increased significantly from 95 (SEM ±2) mmHg to 103 (SEM ±2) mmHg on salt loading ($p<0.0001$). No significant MAP change was reported in normoalbuminuric patients [575]. Similar results were published in microalbuminuric T1DM patients [576]. Indeed, available evidence suggests that salt sensitivity of blood pressure (defined as a MAP increment > or = 3 mmHg on a salt loading) is least common in non-diabetic subjects (17%), increasing to 37% in normoalbuminuric T1DM patients and 50% in T1DM patients with microalbuminuria [577].

Several mechanisms have been put forward to explain salt sensitivity in T2DM patients. These include low prevalent renin concentrations [578-580], hypertension, activated sympathetic nervous system and hyperinsulinism [575, 581-585]. Additionally, evidence points to two types of hereditary SSBP, namely the low renin (LR) phenotype and non-modulation. Whereas patients with the former are characterised by a blunted rise in plama renin activity in response to salt restriction [586], the latter typically display a muted aldosterone response to exogenous angiotensin II despite a normal renin response to a low sodium balance [587, 588]. Underwood et al. reported that elevated BP is the strongest predictor of SSBP in T2DM patients, and that the latter is largely driven by non-modulation [589]. T2DM patients recruited into this exploratory clinical study were normoalbuminuric and

exhibited relatively well controlled blood pressure readings despite withdrawal of their antihypertensive therapy. This, coupled with the small number of participating subjects, lessened the possibility of identifying salt-sensitive patients in either thiazolidinedione subgroup.

Both thiazolidinedione subgroups decreased their DBP in response to chronic salt loading, with the degree of reduction being greater among subjects prone to thiazolidinedione-associated oedema. Ventricular-arterial stiffening is characteristically accompanied by a reduction in DBP. As peripheral arterial resistance increases in older individuals (aged 20 to 70), expanded artery walls are less likely to recoil in diastole, leading to earlier wave reflection, higher SBP, lower DBP, increased pulse pressure and an increase in cardiac afterload, with resultant ventricular-vascular uncoupling [590, 591]. The left ventricle becomes progressively stiffer (possibly an adaptive mechanism) and later hypertrophic, a phenomenon associated with increased cardiovascular risk [592-595]. Extrapolating from this study's exploratory data, a greater reduction in DBP in response to chronic salt loading among thiazolidinedione intolerant patients is consistent with this study's reported greater increase in cAI and pAI, suggesting that such patients are more prone to increase their arterial stiffness on chronic salt exposure.

Exploratory data suggest no consistent differences in TBW between thiazolidinedione categories following a five-day high salt diet, albeit a higher FMI among patients prone to fluid overload. AFV tended to increase in response to both acute and chronic salt loading among tolerant subjects, but decreased following acute intravenous saline infusion in 'intolerant' patients. The degree of increase in AFV

seems to become mitigated in tolerant patients as they progress from acute to chronic salt loading. However, the small number of participating subjects and wide 95% CIs hamper definitive conclusions in this regard. Moreover, TZD intolerant patients seemingly exhibited inconsistent degrees of %AFV shifts in response to chronic salt loading.

Both thiazolidinedione intolerant patients tended to be characterised by higher GFR on salt loading. Subject 10's GFR was higher than that for her 'tolerant' counterparts in response to acute salt loading, whereas subject 11 exhibited a similar trend following a five day high sodium diet. Percentage increase in GFR was generally higher for acute than for chronic salt loading, except for thiazolidinedione intolerant subject 11, where the reverse was true. Lithium ions are freely filtered at the glomerulus and reabsorbed at the proximal tubule in the same proportion as sodium and water. There could be some reabsorption of lithium in the loop of Henle in some extreme conditions [596]. This renders calculation of FeLi a valuable marker of proximal tubule salt and water handling. Thus, a higher FeLi would be consistent with less proximal tubule sodium and water reabsorption, and hence greater delivery to the distal tubules [596]. TZD intolerant subject 10 was characterised by a higher FeLi (implicating greater sodium and water delivery to the distal tubules) albeit no difference in FRDDNa on acute salt loading. Not surprisingly, this translated into a higher FeNa, implicating that this patient improved her natriuresis in response to acute salt loading. Following exposure to a five-day high sodium diet, subject 10's FeLi and FENa were higher, while FRDDNa was lower, again implicated better renal sodium handling than her 'oedema free' counterparts. Thiazolidinedione intolerant subject 11's FRDDNa was no different from that of thiazolidinedione tolerant

patients on acute salt loading. However, this patient was characterised by a lower FENa (less natriuresis) and a lower FeLi, implicating a greater degree of proximal tubular sodium and water reabsorption. In response to chronic salt loading, subject 11 exhibited a higher FeLi (implicating more sodium and water delivery to the distal tubules), albeit no differences in FeNa and FRDDNa, suggesting impaired renal sodium and water handling between the proximal and distal tubules.

In summary, the limited exploratory data for thiazolidinedione intolerant subjects suggest heterogeneity in sodium handling. Subject 11's results are generally consistent with those reported by Zanchi et al. [322], with a role for aquaporins (AQP) 1 and 7, the type 3 sodium hydrogen exchanger (NHE3) or the type 1 sodium-bicarbonate cotansporter in the aetiopathogenesis of thiazolidinedione-associated oedema. On the other hand, subject 10 exhibited a better natriuresis in response to chronic salt loading with an increase in ANP (and possibly BNP) in the context of apparent suppression of renin (and possibly aldosterone); other as yet unidentified mechanisms play a role in fluid overload in her case.

This is the first case-control study comprehensively investigating physiological differences between patients tolerant to thiazolidinediones and those developing HF and/or oedema within three months of their index thiazolidinedione exposure. The study design seemed ideally suited to investigate what was recognised as being a relatively infrequent adverse event (as confirmed in this thesis' population based study in Chapter 3). A case-control design thus permitted a detailed characterisation of both thiazolidinedione subgroups (over three study visits cumulatively lasting several hours), as exposed to different degrees of salt loading. Moreover, a case-

control approach allowed detailed investigation of an adverse event arising from a class of drugs whose prescription has diminished over the years for reasons discussed elsewhere. This study's approach did not permit a calculation of incidence (absolute risk). However, this issue was specifically tackled at a population level elsewhere in this thesis (chapter 3). Ultimately, the main problem encountered was difficulty in identifying adequate numbers of confirmed cases of TZD-intolerant patients for formal statistical analysis, despite comprehensive searching using multiple methods. It is recognised that study design may also have been hampered by selection bias and reliance on recall of exposure to the drug of interest (rosiglitazone/pioglitazone), particularly with respect to the temporality of adverse drug reactions. However, this possibility was inherently minimised by cross-checking with prescription data readily linked to the SCI-DC database. Access to detailed clinical records at NHS Tayside permitted the inclusion of patients who fitted very strict inclusion and exclusion criteria, minimising confounding factors and major biases, particularly when selecting the control group of thiazolidinedione tolerant subjects.

Chapter 3

Factors predicting diuretic prescription and heart failure after initiation of thiazolidinedione therapy

A population based approach

Chapter 3 - Factors predicting diuretic prescription and heart failure after initiation of thiazolidinedione therapy

A population based approach

Section I - Methods

3.1 Rationale of this study

Given the difficulties in identifying thiazolidinedione intolerant patients for the aforementioned clinical study, I embarked on related secondary research based on anonymised person-specific data sets captured by the NHS and the University of Dundee, and managed by the Health Informatics Centre (HIC) at the latter institution. This enabled the identification and characterisation of patients exposed to thiazolidinediones and compared data with two control populations, namely (i) a metformin-sulphonylurea combination therapy cohort, comprising patients treated with established, cheap and effective first and second line oral glucose lowering agents, and (ii) insulin-treated cohort, comprising patients at a more advanced stage of their disease process. There is currently paucity of data comparing incident HF and ‘oedema’ rates between patients treated with index metformin-sulphonylurea combination therapy and index thiazolidinedione therapy. Moreover, it is unclear whether risk factors for incident HF /oedema are shared by patients in either cohort.

3.2 Research aims

This population based research project was designed with the following objectives in mind:

- Defining T2DM at a population level
- Defining incident thiazolidinedione use
- Defining comparator T2DM populations
- Defining index loop diuretic prescription as a surrogate marker of fluid overload /oedema
- Defining incident HF
- Phenotypic characterisation of thiazolidinedione-treated patients.
- Identification and phenotypic characterisation of patients whose index thiazolidinedione therapy was complicated by index loop diuretic prescription and / or congestive HF, and comparing them with their loop diuretic / congestive HF free counterparts
- Defining the genetic characteristics of T2DM patients whose thiazolidinedione treatment was followed by incident loop diuretic use and /or hospitalization for HF
- Comparing the genotypic characteristics of T2DM patients of patients whose treatment with thiazolidinediones was/was not followed by index loop prescription and/or diagnosis of congestive HF

3.3 Hypotheses

This study aimed to investigate the hypotheses that thiazolidinedione-treated patients are at a higher risk of progressing to index loop diuretic prescription (a surrogate marker of oedema) and/or HF compared with patients on 'established' first and second line oral glucose lowering agents (metformin-sulphonylurea combination therapy). Additionally, this study hypothesised that such patients are more likely to progress to such adverse events if they fulfill one or more of the following baseline criteria:

- macrovascular disease
- co-administration of insulin and thiazolidinediones
- non-steroidal anti-inflammatory agents (NSAIDS) and/or dihydropyridine calcium channel blockers
- higher mean systolic blood pressure, higher mean DBP and higher mean arterial pressure (as surrogate markers of arterial stiffness)
- impaired renal function
- impaired left ventricular function
- CYP2C8*1/*1 (wild type) carriers compared with CYP2C8*3 and / or CYP2C8*4 allelic variants

3.4 Study outcomes

Based on the results of epidemiology data, this study sought to explore simple clinical differences between individuals who are ‘tolerant’ and ‘intolerant’ to thiazolidinedione therapy, using a comparative approach. Two cohorts, comprising

metformin-sulphonylurea combination therapy treated patient' and insulin-treated patients (defined in section 3.5) acted as control populations in this regard.

Initially, the primary and secondary outcomes of this study were defined as time from index thiazolidinedione pharmacotherapy to index loop diuretic prescription and incident HF respectively. Acting upon available data, a decision was subsequently made to pursue a *post-hoc* analysis which amalgamated the metformin-sulphonylurea and thiazolidinedione cohorts. This enabled inclusion of index TZD therapy (vs index metformin-sulphonylurea combination therapy) as a covariate in multivariate logistic and Cox regression analyses.

3.5 Study population

This observational cohort study was carried out among the resident population of the Tayside Health Board, Scotland (approximately 400, 000 people). Data were provided by the Health Informatics Centre (HIC), University of Dundee after approval by the Tayside Committee Medical Research Ethics. HIC has developed a record-linkage of multiple routinely-collected datasets to carry out anonymized health-related research in Tayside. Accurate electronic linkage was facilitated by the widespread use of a nine-digit Community Health Identifier that is assigned to all patients in Scotland who are registered with a general practitioner. Data-sets used for this study included:

- Scottish Care Information-Diabetes Collaboration (SCI-DC): a validated population based diabetes clinical information system. The original Diabetes

Audit and Research Tayside (DARTS) database for Tayside has 95% sensitivity for identifying people with diabetes [437].

- Additionally, patients were identified from an ongoing study of the Genetics of Diabetes Audit and Research Tayside (GoDARTS). Since October 1997, all patients with diabetes have been invited to give written informed consent to have their DNA and serum collected as part of the Wellcome Trust United Kingdom Type 2 Diabetes Case Control Collection [597]. As of June 2009, more than 8,000 individuals have participated in the Go-DARTS study [598].

- Scottish Morbidity Record (SMR) data: Forming part of a national database managed by ISD Scotland on behalf of NHS Scotland, the SMR project compiles a comprehensive core data-set based on a standard set of data definitions and codes for the key areas of (i) patient identification and demographic data, (ii) episode management data, and (iii) general clinical data [599]. SMR data were used to identify patients who have been registered with a clinical diagnosis of HF.

- The Tayside echocardiography database: Maintained by the Department of Cardiology at Ninewells Hospital, Dundee, this database hosts all elective outpatient echocardiograms carried out by British Society of Echocardiography (BSE) accredited echocardiographers [600]. A random blinded re-reading of left ventricular functional assessment recorded a 90% concordance rate between results reported in the database and those recorded at independent review [600].

- The above data were linked to the Medicines Monitoring Unit (MEMO) database [499]. The MEMO database was developed for pharmacoepidemiological research in the population of Tayside and contains detailed records of all prescription items dispensed to patients at community pharmacies. Thus for all Tayside patients, there are detailed records of all prescriptions dispensed for thiazolidinediones, insulin, diuretics, and all other drugs referred to hereafter.

This data-linkage permitted a detailed retrospective phenotypic, genetic and pharmacoepidemiological comparison of 'thiazolidinedione intolerant' with 'thiazolidinedione tolerant' cohorts, and with a control population of T2DM patients.

3.5.1 Type 2 diabetes definition

Patients were defined as suffering from T2DM if they were diagnosed after the age of 40, with no progression to insulin within six months of diagnosis, and currently treated with metformin and /or a sulphonylurea. Patients diagnosed above the age of 90 were excluded. Patients commencing insulin more than six months after the diagnosis were eligible for inclusion. This T2DM definition has been adopted and validated elsewhere [601].

3.5.2 Type 2 diabetes cohorts

(i) Thiazolidinedione cohort: a cohort of T2DM patients commenced on a thiazolidinedione (pioglitazone or rosiglitazone) in routine clinical care. This cohort

was further subdivided into patients whose pioglitazone or rosiglitazone therapy was being used (i) in the absence of insulin i.e. as add on to metformin and /or sulphonylurea or as monotherapy and (ii) in combination with insulin (+/- metformin and/or sulphonylurea). Patients treated with adjunct acarbose, nateglinide / repaglinide, sitagliptin / vildagliptin or exenatide / liraglutide (BNF section 6.1.2.3) while on pioglitazone or rosiglitazone were excluded from this cohort.

Patients were eligible for inclusion from the date of index prescription of pioglitazone or rosiglitazone until the date of the last thiazolidinedione prescription/censor unless excluding factors came into effect. Patients were excluded from the thiazolidinedione cohort if they had received any treatment with thiazolidinediones (pioglitazone or rosiglitazone) at any point within the previous twelve months. The index date of thiazolidinedione prescription was defined as the date of first thiazolidinedione prescription which was followed by a subsequent thiazolidinedione prescription within the first three months. If the latter gap exceeded three months, the next eligible thiazolidinedione prescription for inclusion as an index thiazolidinedione prescription was one which had not been preceded by an earlier thiazolidinedione prescription over the previous 12 months.

Thiazolidinedione-treated patients were censored if they commenced treatment with another oral glucose lowering agent, namely acarbose, nateglinide / repaglinide, sitagliptin / vildagliptin or exenatide / liraglutide (BNF sections 6.1.2.3) after index thiazolidinedione prescription. The censor date in this case was defined by the date of first prescription of the first additional oral glucose lowering agent. Thiazolidinedione-treated patients who had been treated with insulin prior to index

thiazolidinedione prescription, but whose insulin was stopped prior to index thiazolidinedione prescription were excluded from the thiazolidinedione cohort. Thiazolidinedione-treated patients who were treated with insulin both before and after index thiazolidinedione prescription were defined as belonging to the TZD + insulin group for the purposes of this study. The minimum number of insulin prescriptions required for inclusion into the TZD + insulin group was set at two - one prescription before index thiazolideindione therapy and one after.

Thiazolidinedione-treated patients who had insulin added on to prevalent thiazolidinedione therapy, and whose thiazolidinedione therapy was continued uninterruptedly were defined by two study dates:

- study period 1 comprising the time between index thiazolidinedione prescription (index date 1) and index insulin prescription. Such patients were included in the TZD – insulin group for the purpose of this study.

- study period 2 comprising the time between the first thiazolidinedione prescription occurring after index insulin prescription (index date 2) and the last thiazolidinedione prescription. Such patients were included in the TZD + insulin group for the purposes of this study.

Thiazolidinedione-treated patients who were commenced on insulin at some point after index thiazolidinedione prescription, and whose thiazolidinedione therapy was stopped at that point were included in the TZD-insulin group.

(ii) Metformin and sulphonylurea combination therapy cohort (MFSU cohort or control cohort 1): a cohort of T2DM patients treated with a combination of metformin and sulphonylurea therapy. This included patients who had a sulphonylurea added on to metformin monotherapy and patients who had metformin added on to sulphonylurea monotherapy.

This cohort excludes treatment with thiazolidinediones at any time point. Patients were also excluded if they were treated with insulin (BNF sections 6.1.1.1 and 6.1.1.2), acarbose, nateglinide / repaglinide, sitagliptin / vildagliptin or exenatide / liraglutide (BNF section 6.1.2.3). Censor date was defined by the date of first prescription of any of these drug or drugs (while on metformin-sulphonylurea combination), whichever was introduced first

The index date of metformin prescription was defined as the date of first metformin prescription which was followed by a subsequent metformin prescription within the first three months. If the latter gap exceeded three months, the next eligible metformin prescription for inclusion as an index metformin prescription was one which had not been preceded by an earlier metformin prescription over the previous 12 months.

The index date of sulphonylurea prescription was defined as the date of first sulphonylurea prescription which was followed by a subsequent sulphonylurea prescription within the first three months. If the latter gap exceeded three months, the next eligible sulphonylurea prescription for inclusion as an index sulphonylurea

prescription was one which had not been preceded by an earlier sulphonylurea prescription over the previous 12 months.

Patients who separately fulfilled index date criteria for metformin and sulphonylurea prescription, as defined above, and whose index dates for metformin and sulphonylurea prescription overlapped, were eligible for inclusion into control cohort 1. Index date for inclusion into this combination control cohort 1 was defined as the first day of adjunct index metformin/sulphonylurea prescription. End date for inclusion into control cohort 1 was defined as the date of the last metformin or sulphonylurea prescription, whichever was withdrawn first. Patients with an index date prior to 1st January 1994 were excluded from inclusion into the cohort.

Patients were likewise censored if commenced on insulin (BNF sections 6.1.1.1 and 6.1.1.2), acarbose, nateglinide / repaglinide, sitagliptin / vildagliptin or exenatide / liraglutide (BNF section 6.1.2.3) after index date. Censor date was defined by the date of first prescription of any of these drug or drugs, whichever was introduced first.

(iii) Insulin-treated cohort (control cohort 2): a cohort of insulin treated T2DM patients treated with insulin

- in combination with metformin and /or sulphonylurea **OR**
- Monotherapy

but excluding thiazolidinedione therapy (pioglitazone or rosiglitazone)

Patients were eligible for inclusion from the date of index insulin prescription until the date of the last insulin prescription/censor unless excluding factors come into effect. To be eligible for inclusion into the control cohort 2, patients must not have had any treatment with insulin within 12 months prior to index insulin prescription

The index date of insulin prescription was defined as the date of first insulin prescription which was followed by a subsequent insulin prescription within the first three months. If the latter gap exceeded three months, the next eligible insulin prescription for inclusion as an index insulin prescription was one which had not been preceded by an earlier insulin prescription over the previous 12 months.

Patients were excluded from this cohort if their index insulin prescription date occurred prior to 1st January 1994.

3.6 Defining drug dose

Thiazolidinedione therapy Population based drug dispensing records were used to express each prescribed dose of thiazolidinedione as a percentage of the maximal prescribed dose in the British National Formulary, deriving a mean percentage dose for each thiazolidinedione-treated patient.

3.7 Definition of heart failure

Individuals were defined as suffering from congestive HF if they fulfil one of the following criteria:

- have had a standardized morbidity record (SMR) for congestive HF. This was defined as a hospital admission International Classification of Diseases, Ninth Revision and 10th Revision (ICD 9/10) diagnostic code for congestive HF during the study period (ICD-9 code 428, ICD-10 code 150). The date of admission was defined as the date of CHF diagnosis. An SMR for HF in Tayside gives the date of admission, type of admission (emergency or not), and the primary reason for admission according to the ICD code.

OR

- have had echocardiographic evidence of left ventricular systolic dysfunction and prescription of a loop diuretic (BNF code 2.2.2) within one year. The date of prescription of a loop diuretic and/or diagnosis of left ventricular systolic dysfunction, whichever came first, was defined as the date of CHF diagnosis. The latter echocardiographic based definition of HF has been validated elsewhere (reporting a 91% concordance with a clinical diagnosis of HF from case note review).

Any subsequent CHF events after diagnosis date were defined using SMR data.

3.8 Clinical data extraction

3.8.1 Basic demographics

Basic demographic criteria captured within this dataset included index date of inclusion and the date until which individual patients satisfied the aforementioned set criteria for inclusion into their treatment cohort. This approach permitted calculation of duration of metformin-sulphonylurea combination, insulin or thiazolidinedione therapy for each individual patient. Duration of T2DM was defined by the number of days elapsed since diagnosis of T2DM at inclusion into the respective treatment cohort. Age (in years) and gender were likewise captured at inclusion into the cohort (table 3.1).

Table 3.1 - Baseline demographics

Clinical characteristic	*Units*	*Definition*
Age	years	Age at inclusion into the cohort
Gender	-	Male/female
Duration of type 2 diabetes	days	days elapsed since registered diagnosis of type 2 diabetes at inclusion into the respective cohort
Duration of treatment	days	days elapsed between inclusion into the respective cohort and date until which patient satisfied criteria for inclusion into the cohort

3.8.2 Past medical history

Baseline and post-treatment past medical history (coronary artery disease, stroke, peripheral artery disease) were defined by ICD coding for the respective event prior

to, and after, inclusion into either of the three treatment cohorts respectively (table 3.2). Additionally, a macrovascular composite (baseline/post-treatment) was generated from this dataset, encompassing the occurrence of either of these three events before or after index metformin-sulphonylurea, insulin or thiazolidinedione prescription.

Table 3.2 - Past medical history

Past medical history	***Definition***
Coronary artery disease	ICD 10:120-125, ICD 9:410-414
Stroke	ICD 10:160-169, ICD 9:430-438
Peripheral artery disease	ICD 10:1739, ICD 9:4439

3.8.3 Drug history

Individual drug therapy was defined by the respective drug's BNF code, as outlined in table 3.3 below. Baseline and post-treatment drug therapy were defined by capturing evidence of a prescription prior to, and after, inclusion into the respective treatment cohort respectively.

Table 3.3 - Drug history

Drug history	*Definition*
Peripheral vasodilators and related drugs	BNF code 2.6.4
Thiazide diuretics	BNF code 2.2.1
Loop diuretics	BNF code 2.2.2
Potassium sparing diuretics / aldosterone antagonists	BNF code 2.2.3
Non-steroidal anti-inflammatory drugs	BNF code 10.1.1
Dihydropyridine calcium channel blockers	Amlodipine, felodipine, isradipine, lacipidine, lercanadipine, nicardipine, nifediipine or nimodipine
Verapamil	
Diltiazem	
Beta-adrenoceptor blocking drugs	BNF code 2.4
Vasodilator antihypertensive drugs	BNF code 2.5.1
Centrally acting antihypertensive drugs	BNF code 2.5.2
Adrenergic neurone blocking drugs	BNF code 2.5.3
Alpha adrenoceptor blocking drugs	BNF code 2.5.4
Angiotensin-converting enzyme inhibitors	BNF code 2.5.5.1
Angiotensin-II receptor antagonists	BNF code 2.5.5.2
Renin inhibitors	BNF code 2.5.5.3
Nitrates	BNF code 2.6.1
Other antianginal drugs	BNF code 2.6.3

3.8.4 Clinical measurements

Given the likely fluctuant nature of blood pressure readings, baseline SBP and DBP were defined as mean values measured in the year prior to prescription of the index glucose lowering drug(s) of interest (table 3.4). Post-treatment SBP and DBP were defined by the mean of any readings measured within the first year (excluding readings taken less than 30 days) after inclusion into the cohort. In contrast, as weight changes are likely to be more progressive and sustained, baseline and post-treatment weight and BMI measurements were defined by the respective closest values before, and at least 30 days after, prescription of index metformin-sulphonylurea combination, insulin or thiazolidinedione therapy.

Table 3.4 - Clinical measurements

Clinical measurements	*Units*	*Definition*
Systolic blood pressure	mmHg	Mean values for the year before, 30-365 days after, inclusion [a]
Diastolic blood pressure	mmHg	Mean values for the year before, 30-365 days after, inclusion [a]
Weight	kg	Closest values before, 30-365 days after, inclusion [a]
Body mass index	kg/m^2	Closest values before, 30-365 days after, inclusion [a]

[a] *into the respective treatment cohort*

3.8.5 Laboratory investigations

Likewise, baseline and post-treatment values for basic laboratory investigations were captured from routine clinical measurements (table 3.5). Baseline values were defined by the most recent result issued prior to inclusion into the respective treatment cohort. Post-treatment laboratory investigation values were defined by the earliest result issued at least 30 days after inclusion, with the exception of post-treatment HbA1c, defined as the earliest value measured between 30 days and 18 months after recruitment into the metformin-sulphonylurea combination, insulin or thiazolidinedione cohort. Estimated glomerular filtration rate (eGFR) values (reported in mls/min/1.73 m^2) were calculated from available age, weight and serum creatinine values using the established Cockcroft-Gault formula [602]:

(140 – age) * lean body mass / plasma creatinine * 72 for males, and

[(140 – age) * lean body mass / plasma creatinine * 72] * 0.85 for females

Table 3.5 - Laboratory investigations

Laboratory investigations	*Units*	*Definition*
Haematocrit	%	Most recent value prior to, at least 30 days after, inclusion [a]
HbA1c	%	Most recent value prior to, 30 days – 18 months after, inclusion [a]
TC	mmol/L	Most recent value prior to, at least 30 days after, inclusion [a]
HDL-C	mmol/L	Most recent value prior to, at least 30 days after, inclusion [a]
LDL-C	mmol/L	Most recent value prior to, at least 30 days after, inclusion [a]
Triglycerides	mmol/L	Most recent value prior to, at least 30 days after, inclusion [a]
ALT	IU/L	Most recent value prior to, at least 30 days after, inclusion [a]
Sodium	mmol/L	Most recent value prior to, at least 30 days after, inclusion [a]
Creatinine	μmol/L	Most recent value prior to, at least 30 days after, inclusion [a]
eGFR	mls/min/1.73m^2	Most recent value prior to, at least 30 days after, inclusion [a]
Albumin	g/L	Most recent value prior to, at least 30 days after, inclusion [a]
TSH	IU/L	Most recent value prior to, at least 30 days after, inclusion [a]

[a] *into the respective treatment cohort; ALT, alanine aminotransferase; eGFR, estimated glomerauld filtration rate; HbA1c, glysoylated haemoglobin; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; TSH, thyroid stimulating hormone*

3.8.6 Echocardiography measurements

Likewise, this study captured echocardiographic measurements from recruited T2DM patients who had undergone tissue Doppler echocardiography (table 3.6). Baseline and post-treatment measurements were defined as the most recent values measured prior to, and at least 30 days after inclusion into their respective treatment cohort. Intraventricular septum width and left ventricular posterior wall thickness were measured at end-diastole.

Left ventricular mass (LVM) was defined as

$$\mathbf{0.8\ (1.04[(LVID + LVPW + IVS)^3 - (LVID)^3]) + 0.6g}$$

as conventionally defined by Devereux et al. [603], validated at necropsy ($r = 0.90$; $p < 0.001$) [604], and endorsed by the American Society of Echocardiography [605], where LVID denotes left ventricular internal diameter at diastole, LVPW thickness denotes left ventricular posterior wall thickness at end-diastoleand IVS thickness denotes intra-ventricular septum thickness at end-diastole.

Table 3.6 - Echocardiography measurements

Echocardiography measurements	***Units***	***Definition***
IVS thickness	cm	Most recent values measured [a] prior to, at least 30 days after, inclusion [b]
LVPW thickness	cm	Most recent values measured [a] prior to, at least 30 days after, inclusion [b]
LV mass	g	Most recent values measured prior to, at least 30 days after, inclusion [b]

[a] *measured at end-diastole;* [b] *into the respective cohort; IVS, interventricular septum; ; LV, left ventricular; LVPW, left ventricular posterior wall*

3.8.7 Genotyping

Genotyping of CYP2C8*3 and CYP2C8*4 variants was carried out under the manufacturer's (Applied Biosystems) recommended standard conditions using Taqman-based allelic discrimination assays. The overall genotyping call rate was

94% and both SNPs were in Hardy-Weinberg Equilibrium in the sample ($p > 0.05$). Genotyping data were extracted and merged with the available datasets for analysis.

3.9 Statistical methods

3.9.1 Descriptive statistics

Continuous variables were expressed as means and standard deviations. Dichotomous variables were expressed as percentages. Continuous variables did not satisfy criteria for normality (as assessed by visual plot inspection and estimation of skewness) were transformed ($\log_e$, square root or reciprocal) to achieve normality. Between-group differences across normally distributed variables were compared using one-way Analysis of Variance (ANOVA). Skewed variables which defied attempts at normalisation through transformation were compared using the Mann-Whitney U test. Chi Square and Fisher's exact tests were used to compare dichotomous variables. All tests were two-sided, with a p value < 0.05 considered as statistically significant. All *post-hoc* analyses were Bonferroni, Tukey-HSD or Games-Howell test corrected, as appropriate. Statistical analyses were performed using IBM Social Package for the Statistical Sciences (SPSS®) version 18.0.

3.9.2 Logistic regression analysis

Binary logistic regression analyses were conducted to predict (i) index loop diuretic prescription, and (ii) incident HF within one year after exposure to metformin-sulphonylurea combination / thiazolidinedione therapy. The backward:LR regression

method was used in each case. The regression of the binary outcome (index loop diuretic prescription / incident HF) on the covariates included only those covariates passing the univariable screening. Binary univariate logistic regression was thus run between index loop diuretic prescription / incident HF (dependent variable) and individual continuous and categorical variables (individually acting as independent variables), separating the covariates into those significant and those not significant at $p < 0.1$. Categorical covariates were dummy coded, using non-exposure to the categorical variable of interest as the reference group (and conversely, exposure as the indicator group). Index thiazolidinedione prescription (vs metformin-sulphonylurea combination) was included as a covariate in the logistic regression models, irrespective of the outcome of its univariate regression with the dependent categorical variables of interest, in a bid to emphazise its contribution or otherwise in predicting fluid overload/HF events. Logistic regression models were tested for residuals and overdispersion, and satisfied the assumptions of linearity of logit and multicollinearity. ROC curves were generated for each model to assess model discrimination.

3.9.3 Time to event analysis

Cox proportional hazards regression models (Backward:LR method) were used to predict (i) time to index loop diuretic prescription and (ii) time to incident HF within one year of inclusion into the metformin-sulphonylurea combination therapy or thiazolidinedione cohort, investigating (i) predictors of either event of interest and (ii) specifically whether thiazolidinedione prescription (as a categorical covariate) has a significant impact on either outcome.

Binary univariate logistic regression was run between prescription of index loop diuretic within one year of exposure to index metformin-sulphonylurea combination / thiazolidinedione therapy (dependent variable) and individual continuous and categorical variables (individually acting as independent variables), separating the covariates into those significant and those not significant at $p < 0.1$. Univariate regression of continuous variables was carried out using univariate Cox regression while univariate regression of categorical variables was carried out using Kaplan Meier survival analysis (separating significant and non-significant categorical covariates using the Log Rank test). Likewise, binary univariate logistic regression was run between development of incident HF within one year of inclusion into the metformin-sulphonylurea combination / thiazolidinedione cohort and the same individual continuous and categorical covariates (independent variables). Only covariates passing univariate screening ($p < 0.1$) were considered for inclusion into the Cox regression model. Time-independent covariates were included The Proportional Hazards assumption was formally assessed using log-minus-log against survival/log survival time plots. Covariates not satisfying the Proportional Hazards Assumption on account of a time-varying effect were transformed into time-dependent covariates by forming an interaction (product) term between the individual predictor (continuous or categorical) and a function of time ($\log_e$ time to index loop diuretic prescription / incident HF, whichever was applicable), as described by Bellera et al. [606]. Covariates which seemingly satisfied the Proportional Hazards Assumption were nonetheless transformed into time-dependent covariates using the same procedures, in order to confirm their time-independent contribution to the final model. The correlation between any categorical variables

that proved to be significant ($p < 0.1$) at univariate regression (log rank test) was determined using a chi square test. When two variables were significantly correlated, the variable more significantly linked to index loop diuretic prescription / incident HF (and hence to ‘fluid overload’) was included in multivariate analysis.

Section II - Results

3.10 Data capture – number of patients in each treatment cohort

3027 thiazolidinedione-treated T2DM patients potentially fitted the inclusion criteria for this cohort. Of these, 2754 individuals could be assigned an index prescription date. 55 patients were excluded given they were being treated with other antidiabetic drugs, leaving 2699 patients. A further 15 thiazolidinedione-treated patients were excluded as they had commenced and stopped insulin therapy prior to index thiazolidindedione prescription, leaving 2684 patients.

Table 3.7 - Total number N (%) of thiazolidinedione-treated patients fitting the inclusion criteria for this study.

Insulin prescribing definition	*N (%)*	*TZD cohort subtype*
Insulin-naïve	2070 (76.7)	***TZD - insulin group***
Insulin therapy commenced before and continued after index TZD prescription	60 (2.3)	***TZD + insulin group***
Insulin introduced after index thiazolidinedione prescription, followed by cessation of TZDs	475 (17.6)	***TZD - insulin group***
Insulin introduced after index thiazolidinedione prescription, followed by continuation of TZDs	79 (2.9)*	***TZD - insulin group until index insulin prescription. TZD + insulin group after index insulin prescription***

** Only 38 out of these 79 patients had an identifiable index date for TZD prescription after index insulin prescription.*

In summary, the thiazolidinedione cohort comprised 2722 patients (1542 males, 1180 females) ie 2684 patients + the 38 patients with an index date for thiazolidinedione prescription after index insulin therapy.

The TZD-insulin group comprised a total of 2624 patients (1489 males, 1135 females) subdivided into:

- 2070 thiazolidinedione-treated insulin-naïve patients (never treated with insulin)
- 475 patients who had insulin therapy introduced after index thiazolidinedione prescription followed by cessation of thiazolidinedione therapy
- 79 thiazolidinedione-treated patients whose adjunct insulin therapy (introduced after index thiazolidinedione prescription) was accompanied by continuation of thiazolidinedione therapy (censored at first insulin prescription).

TZD + insulin group comprised a total of 98 patients (53 males, 45 females) subdivided into:

- 60 thiazolidinedione-treated patients whose insulin therapy had been commenced before and continued after index thiazolidinedione prescription
- 38 thiazolidinedione-treated patients whose insulin therapy was introduced after index thiazolidinedione prescription, followed by continuation of thiazolidinediones.

The metformin-sulphonylurea cohort comprised a total of 3725 patients (2079 males, 1646 females). 2205 patients (1124 males, 1081 females) were treated with insulin (without thiazolidinediones).

3.10.1 Patients treated with metformin-sulphonylurea combination therapy, insulin and thiazolidinediones in excess of 90 days.

In order to control for confounding variables arising out of poor drug compliance, this study opted to analyze data from patients who were treated with thiazolidinediones for more than 90 days. 2664 thiazolidinedione-treated patients fitted these inclusion criteria (1511 males, 1153 females), of whom 2566 (1458 males, 1108 females) belonged to the TZD – insulin group and 98 (53 males, 45 females) belonged to the TZD + insulin group. A summary of the relative distribution of patients within each treatment group is summarised in table 3.8.

Table 3.8 - Total number N of patients treated in excess of 90 days and fitting the inclusion criteria for this study.

	Metformin-sulphonylurea cohort	***Insulin cohort***	***Thiazolidinedione cohort***
Males and females	3706	2205	2664
Males	2067	1124	1511
Females	1639	1081	1153

1021 (38.3%) patients prescribed pioglitazone at inclusion into the thiazolidinedione cohort, whereas 1643 (61.7%) patients were administered rosiglitazone as their first thiazolidinedione prescription. As patients tend to be switched from rosiglitazone to pioglitazone, or *vice versa*, an attempt was made to capture these prescription trends in the dataset (table 3.9). There were no data to this effect for 2052 (77%) patients [994 (97.36%) pioglitazone and 1058 (64.40 %) rosiglitazone-treated patients]. Only

548 (20.6%) patients had not had their initial thiazolidinedione replaced by another [13 (1.3%) pioglitazone and 535 (32.6%) rosiglitazone-treated patients]. Thus it can be concluded that 535 (20.08 %) of patients were treated with pioglitazone alone during their observation period, whereas only 13 patients (0.49%) received rosiglitazone monotherapy throughout their follow-up period. At least 64 (2.4%) patients switched between the two thiazolidinediones, for reasons which were not captured for the purposes of this study, rendering any ascertainment of drug-specific (as opposed to class-specific) adverse effects difficult and probably imprecise.

Table 3.9 - Total number N (%) of patients treated with thiazolidinediones in excess of 90 days and fitting the inclusion criteria for this study, classified according to tendency to switch between rosiglitazone and pioglitazone therapy.

	Initial pioglitazone prescription (n = 1021)	***Initial rosiglitazone prescription (n = 1643)***
Data unavailable	994 (97.36)	1058 (64.40)
No switch	13 (1.27)	535 (32.56)
Switched between thiazolidinediones	14 (1.37)	50 (3.04)

3706 patients (2067 males, 1639 females) received treatment with metformin and sulphonylureas in combination for more than 90 days, and were thus included in subsequent analysis of index loop diuretic prescription and incident HF events within one year of inclusion into their respective cohorts (table 3.8).

2205 patients (1124 males and 1081 females) were treated with insulin therapy in excess of 90 days, and were thus recruited for further analysis (table 3.8).

Interestingly, this approach did not diminish the original number of insulin-treated patients fitting the inclusion criteria, presumably because insulin is a 'final' therapeutic option in patients with T2DM.

Further analysis will refer to patients treated with metformin-sulphonylurea (MFSU) combination, insulin and thiazolidinedione therapy in excess of 90 days.

3.10.2 Background loop diuretic therapy at inclusion into each respective treatment cohort

As the intention was to compare patients in whom index metformin-sulphonylurea combination therapy, insulin monotherapy or thiazolidinedione therapy was followed by index loop diuretic prescription, it was necessary to exclude patients with a background of loop diuretic therapy at inclusion into their respective cohort. This left a total of 2785 (1634 males, 1151 females), 1361 (744 males, 617 females) and 2097 (1264 males, 833 females) in the metformin-sulphonylurea, insulin and thiazolidinedione cohorts respectively (tables 3.10 and 3.11, figures 3.1 and 3.2).

The corollary to this observation is that 21.3 % of patients (567 out of 2664) were already being treated with a loop diuretic at index thiazolidinedione prescription. The respective proportions for MFSU and insulin-treated patients were 24.9 % and 38.3% respectively (tables 3.10 and 3.11, figures 3.1 and 3.2). Pairwise *post-hoc* comparisons between the cohorts (Bonferroni corrected) confirmed that these differences reached statistical significance (except for male patients prescribed a thiazolidinedione vs metformin-sulphonylurea combination therapy). Although rates

of background loop diuretic therapy for thiazolidinedione therapy were the lowest among the three cohorts, one would have expected a smaller proportion of such ‘fluid overloaded’ patients being prescribed a drug repeatedly associated with weight gain, fluid retention and HF events. Background loop diuretic rates for T2DM patients prescribed insulin pharmacotherapy are not entirely surprising, given that the latter tends to be prescribed at a relatively ‘late’ stage of the disease, in patients prone to other cardiovascular risk factors and/or established coronary artery disease.

Table 3.10 - Differences in frequency of background loop diuretics therapy at inclusion into the metformin-sulphonylurea, insulin and thiazolidinedione cohorts for at least three months.

Gender subgroup	*Metformin-sulphonylurea cohort* *N = 3706 (2067 males 1639 females)*				*Insulin cohort* *N = 2205 (1124 males 1081 females)*				*Thiazolidinedione cohort* *N = 2664 (1511 males 1153 females)*				*p*[a]
	Background loop diuretic prescribed		*Background loop diuretic-free*		*Background loop diuretic prescribed*		*Background loop diuretic-free*		*Background loop diuretic prescribed*		*Background loop diuretic-free*		
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	
Males and females	921	24.85	2785	75.15	844	38.28	1361	61.72	567	21.28	2097	78.72	< 0.001[b]
Males	433	20.95	1634	79.05	380	33.81	744	66.19	247	16.35	1264	83.65	< 0.001[c]
Females	488	29.77	1151	70.23	464	42.92	617	57.08	320	27.75	833	72.25	< 0.001[d]

[a] *Chi square test for the overall difference between metformin-sulphonlylurea, insulin and thiazolidinedione cohorts. Statistical significance is defined by a two-sided p value of < 0.05.*
[b] *Chi Square = 194.055, df = 2*
[c] *Chi Square = 117.917, df = 2*
[d] *Chi Square = 70.338, df = 2*

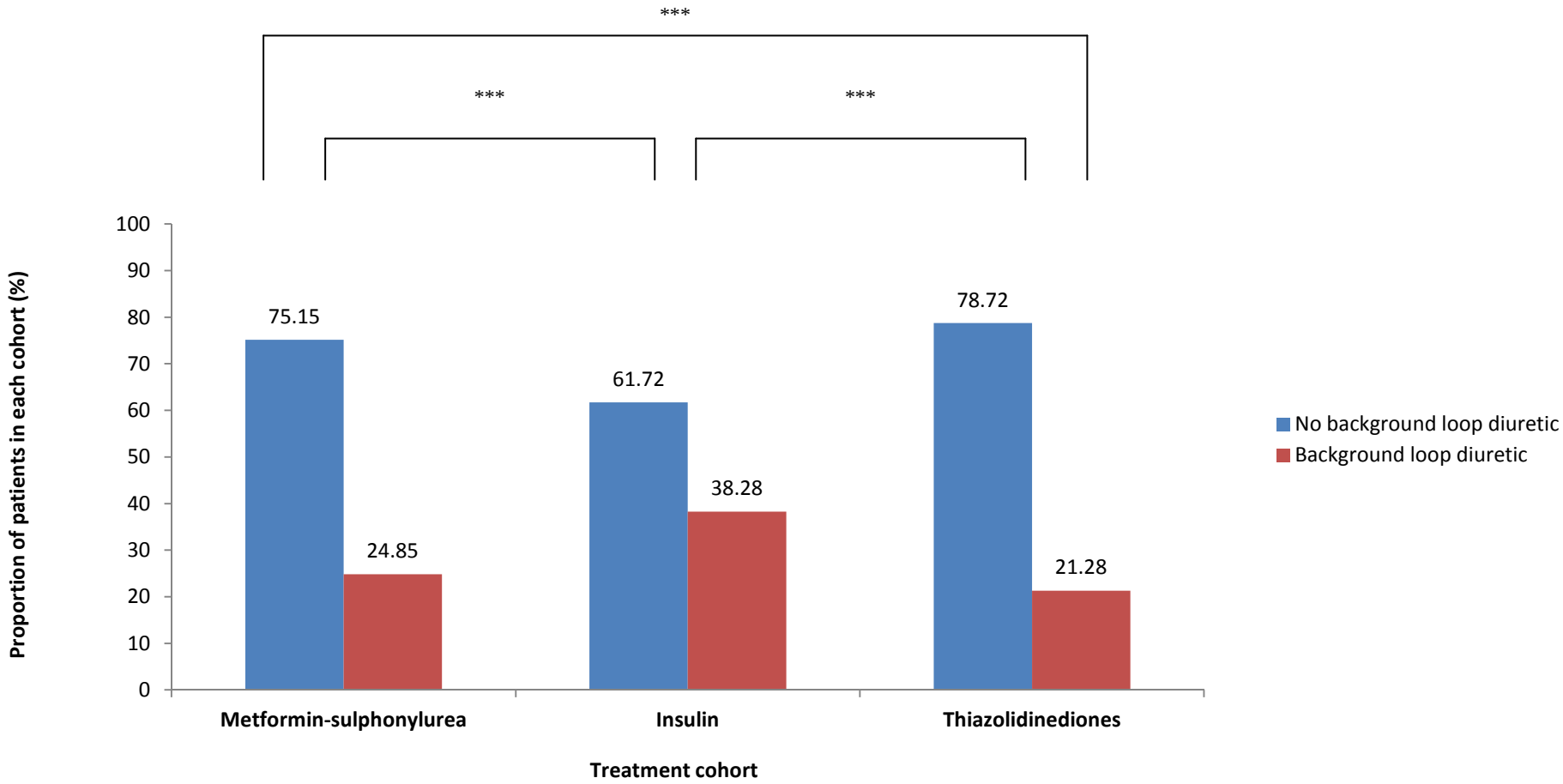
Table 3.11 - Post-hoc analysis: Chi square tests for the association between frequency of background loop diuretic therapy and inclusion into the metformin-sulphonylurea, insulin and thiazolidinedione cohorts for at least three months.

Gender subgroup	*Metformin-sulphonylurea cohort vs insulin cohort*			*Metformin-sulphonylurea cohort vs thiazolidinedione cohort*			*Insulin cohort vs thiazolidinedione cohort*		
	Chi square	*df*	*p*[a]	*Chi square*	*df*	*p*[a]	*Chi square*	*df*	*p*[a]
Males and females	118.969	1	< 0.001	11.020	1	0.001	169.264	1	< 0.001
Males	63.414	1	< 0.001	12.007	1	0.001	108.373	1	< 0.001
Females	49.504	1	< 0.001	1.344	1	0.246	56.364	1	< 0.001

Tests of the three a priori hypotheses were conducted using Bonferroni adjusted alpha levels of 0.0167 per test (0.05/3).

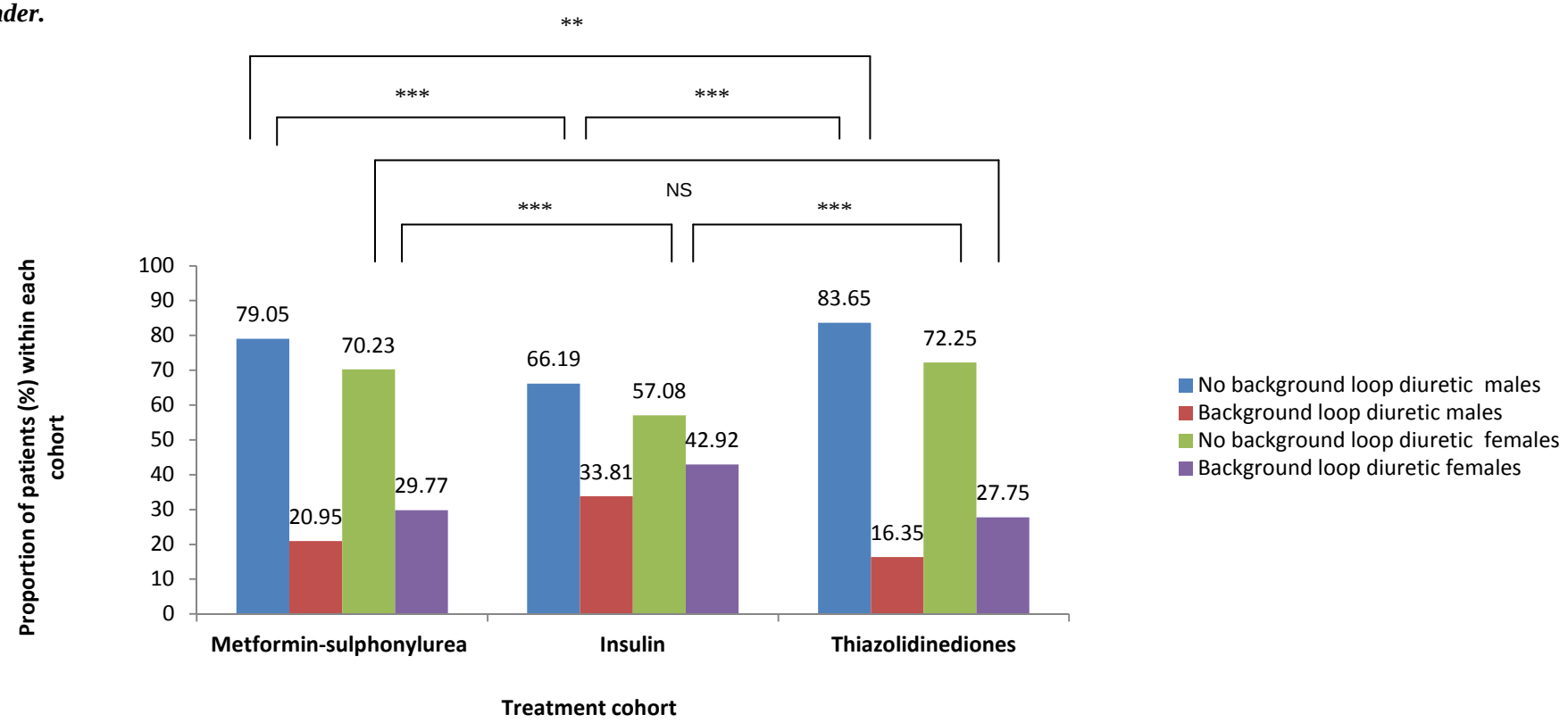
[a] *two-sided p value*

Figure 3.1 - Relative proportions (%) of background loop diuretic therapy at inclusion into each respective cohort for at least three months.



*$p < 0.001$ for the overall difference in loop diuretic treatment counts across the three treatment cohorts; *** $p < 0.001$; the three pairs of post-hoc tests were Bonferroni corrected.*

Figure 3.2 - Relative proportions (%) of background loop diuretic therapy at inclusion into each respective cohort for at least three months, stratified by gender.



*p< 0.001 for the overall difference in loop diuretic treatment counts across the three treatment cohorts; *** p < 0.001; ** p = 0.001; NS, no statistical difference; the three pairs of post-hoc tests were Bonferroni corrected*

3.10.3 Background heart failure at inclusion into each respective treatment cohort

Likewise, this study sought to investigate for differences in rates of HF among thiazolidinedione-treated patients, and patients belonging to the two control cohorts. HF data were derived from SMR, index loop diuretic and echocardiography data, as outlined earlier. The relative proportions of patients identified as suffering from background HF based on these definitions are outlined in table 3.12 below:

Table 3.12 - Derivation of baseline heart failure (HF) data at inclusion into the respective treatment cohort, based on data extraction definitions.

Baseline HF [1] definition	***Metformin-sulphonylurea cohort [4]***	***Insulin cohort [4]***	***Thiazolidinedione cohort [4]***
Echo + loop data [2]			
Males and females	55	93	44
Males	32	63	28
Females	23	30	16
SMR [3] data			
Males and females	175	295	71
Males	102	166	44
Females	73	129	27

[1] HF, heart failure; [2] echo + loop data, echocardiographic evidence of left ventricular systolic dysfunction and prescription of a loop diuretic within one year; [3] SMR, Scottish morbidity record; [4] number of patients captured based on each data extraction definition.

Analyzing for differences in the rates of occurrence of background HF (tables 3.13 and 3.14, figures 3.3 and 3.4), 4.32% of patients were prescribed a thiazolidinedione against a background of HF. The corresponding figures for metformin-sulphonylurea combination and insulin-treated patients were 6.21% and 17.60%, which translates into a significant difference across the three treatment cohorts ($p < 0.001$). Overall, these proportions are consistent with observations reported for background loop

diuretic therapy. Pairwise comparisons (Bonferroni corrected) showed that thiazolidinedione-treated patients had lower rates of background HF compared with their metformin-sulphonylurea combination ($p = 0.001$) or insulin-treated ($p < 0.001$) counterparts. As discussed earlier, the latter observation is likely to represent the end-result of a progressive illness characterised by a tendency to progress to coronary artery disease. One would have expected a lower proportion of patients having thiazolidinediones prescribed against a background of HF, given the much publicized association with fluid overload.

Table 3.13 - Differences in frequency of occurrence of background heart failure at inclusion into the metformin-sulphonylurea, insulin and thiazolidinedione cohorts for at least three months.

Gender subgroup	*Metformin-sulphonylurea cohort* *N = 3706 (2067 males 1639 females)*				*Insulin cohort* *N = 2205 (1124 males 1081 females)*				*Thiazolidinedione cohort* *N = 2664 (1511 males 1153 females)*				*p*[a]
	Background heart failure present		*Background heart failure free*		*Background heart failure present*		*Background heart failure free*		*Background heart failure present*		*Background heart failure free*		
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	
Males and females	230	6.21	3476	93.79	388	17.60	1817	82.40	115	4.32	2549	95.68	<0.001
Males	134	6.48	1933	93.52	229	20.37	895	79.63	72	4.77	1439	95.23	<0.001
Females	96	5.86	1543	94.14	159	14.71	922	85.29	43	3.73	1110	96.27	<0.001

[a] *Chi square test for the overall difference between metformin-sulphonlylurea, insulin and thiazolidinedione cohorts. Statistical significance is defined by a two-sided p value of < 0.05.*
[b] *Chi Square = 317.942, df = 2*
[c] *Chi Square = 220.714, df = 2*
[d] *Chi Square = 108.194, df = 2*

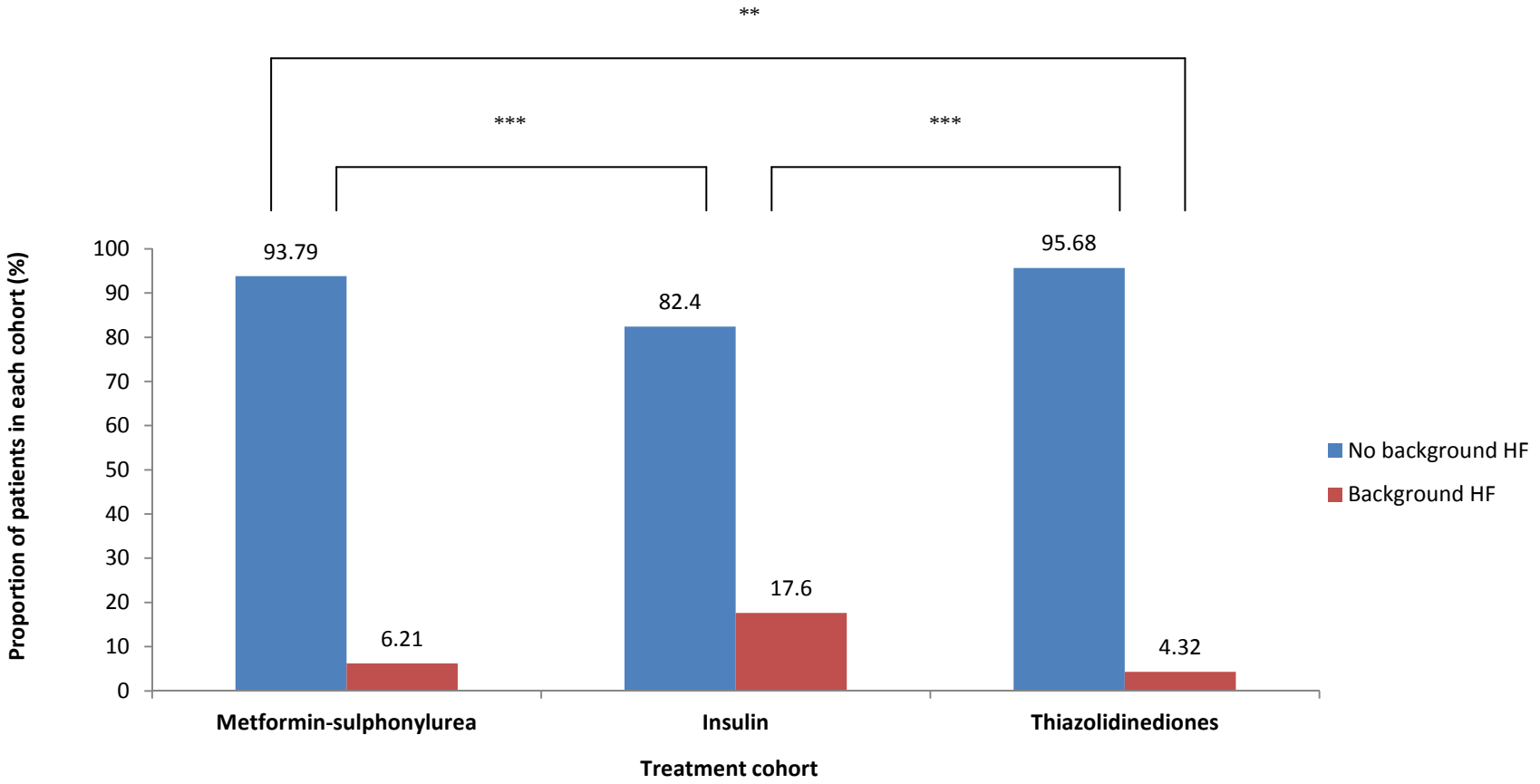
Table 3.14 - Post-hoc analysis: Chi square tests for the association between frequency of occurrence of background heart failure and inclusion into the metformin-sulphonylurea, insulin and thiazolidinedione cohorts for at least three months.

Gender subgroup	*Metformin-sulphonylurea cohort vs insulin cohort*			*Metformin-sulphonylurea cohort vs thiazolidinedione cohort*			*Insulin cohort vs thiazolidinedione cohort*		
	Chi square	*df*	*p*[a]	*Chi square*	*df*	*p*[a]	*Chi square*	*df*	*p*[a]
Males and females	191.579	1	< 0.001	10.800	1	0.001	229.667	1	< 0.001
Males	139.349	1	< 0.001	4.747	1	0.029	155.193	1	< 0.001
Females	60.067	1	< 0.001	6.478	1	0.011	81.772	1	< 0.001

Tests of the three a priori hypotheses were conducted using Bonferroni adjusted alpha levels of 0.0167 per test (0.05/3).

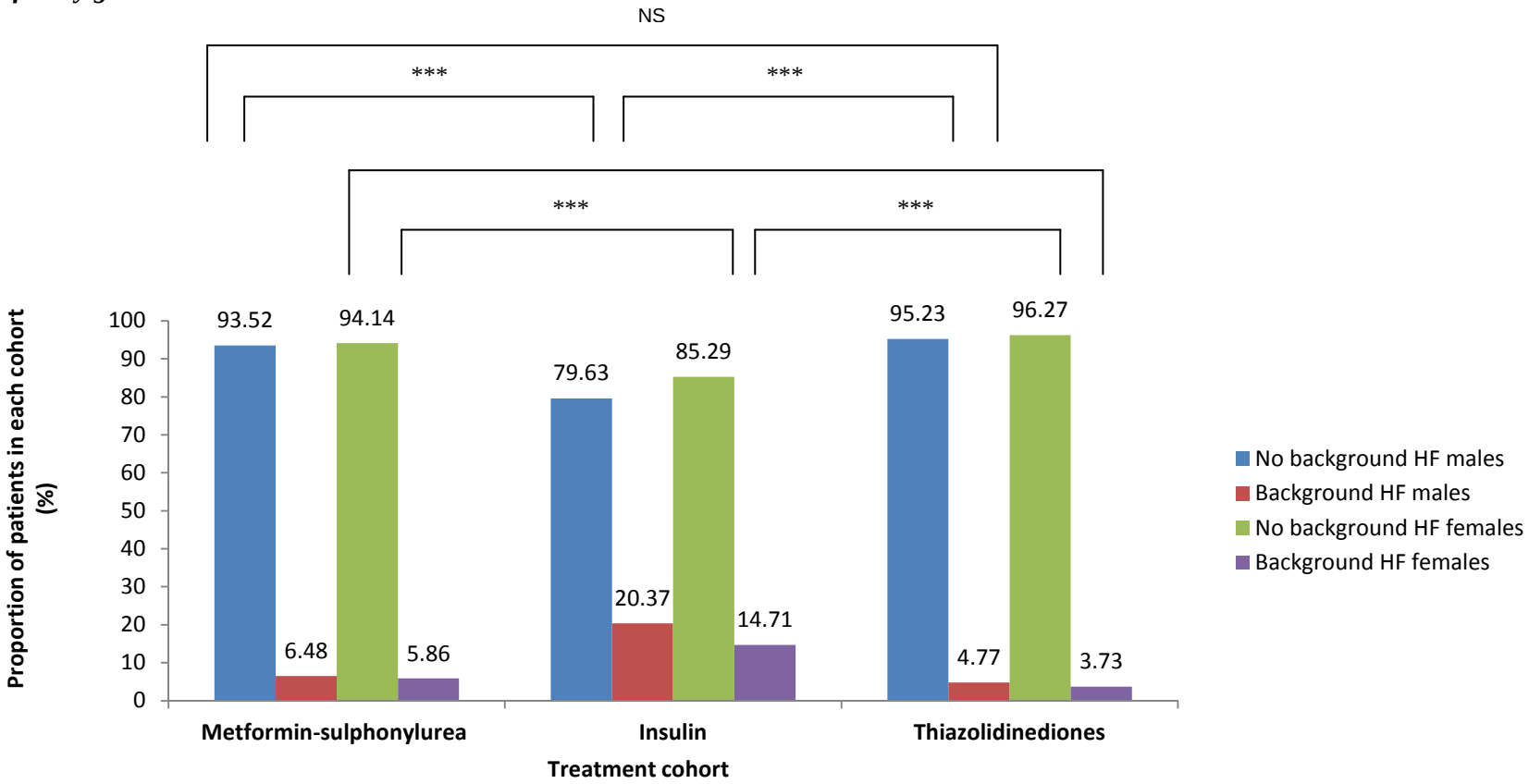
[a] *two-sided p value*

Figure 3.3 - Relative proportions (%) of background occurrence of heart failure (HF) at inclusion into each respective cohort for at least three months.



*p< 0.001 for the overall difference in background heart failure (HF) counts across the three treatment cohorts; *** p < 0.001; ** p = 0.001; the three pairs of post-hoc tests were Bonferroni corrected (statistical significance defined by a p value < 0.0167).*

Figure 3.4 - Relative proportions (%) of background occurrence of heart failure (HF) at inclusion into each respective cohort for at least three months, stratified by gender.



*p < 0.001 for the overall difference in background heart failure counts across the three treatment cohorts; *** p < 0.001; * p = 0.011; NS, NS = no statistical difference; the three pairs of post-hoc tests were Bonferroni corrected (statistical significance defined by a p value < 0.0167)*

3.10.4 Prescription of index loop diuretic therapy within one year of inclusion into each respective treatment cohort

Given the difficulties in controlling for all potential confounding variables that could account for index loop diuretic prescription and incident HF events, this study analysis was limited to events occurring within one year (365 days) after inclusion into the metformin-sulphonylurea, thiazolidinedione and insulin cohorts. Such an approach was more likely to capture this study's drug related adverse effects of interest. 4.3% of patients required an index loop diuretic within one year of their first prescription for rosiglitazone or pioglitazone. This figure was comparable to that for patients on metformin-sulphonylurea combination therapy (4.7%; $p = 0.493$), but significantly lower than for patients commenced on insulin (12.5%; $p < 0.001$) (tables 3.15 and 3.16, figure 3.5). Stratifying by gender yielded similar results (tables 3.15 and 3.16, figure 3.6).

Table 3.15 - Differences in frequency of prescription of index loop diuretics within one year after inclusion into the metformin-sulphonylurea, insulin and thiazolidinedione cohorts for at least three months.

Gender subgroup	*Metformin-sulphonylurea cohort* *N = 2785 (1634 males 1151 females)*				*Insulin cohort* *N = 1361 (744 males 617 females)*				*Thiazolidinedione cohort* *N = 2097 (1264 males 833 females)*				*p*[a]
	Index loop diuretic prescribed within one year		*Index loop diuretic-free within one year*		*Index loop diuretic prescribed within one year*		*Index loop diuretic-free within one year*		*Index loop diuretic prescribed within one year*		*Index loop diuretic-free within one year*		
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	
Males and females	131	4.7	2654	95.3	170	12.5	1191	87.5	90	4.3	2007	95.7	< 0.001 [b]
Males	74	4.5	1560	95.5	81	10.9	663	89.1	40	3.2	1224	96.8	< 0.001 [c]
Females	57	5.0	1094	95.0	89	14.4	528	85.6	50	6.0	783	94.0	< 0.001 [d]

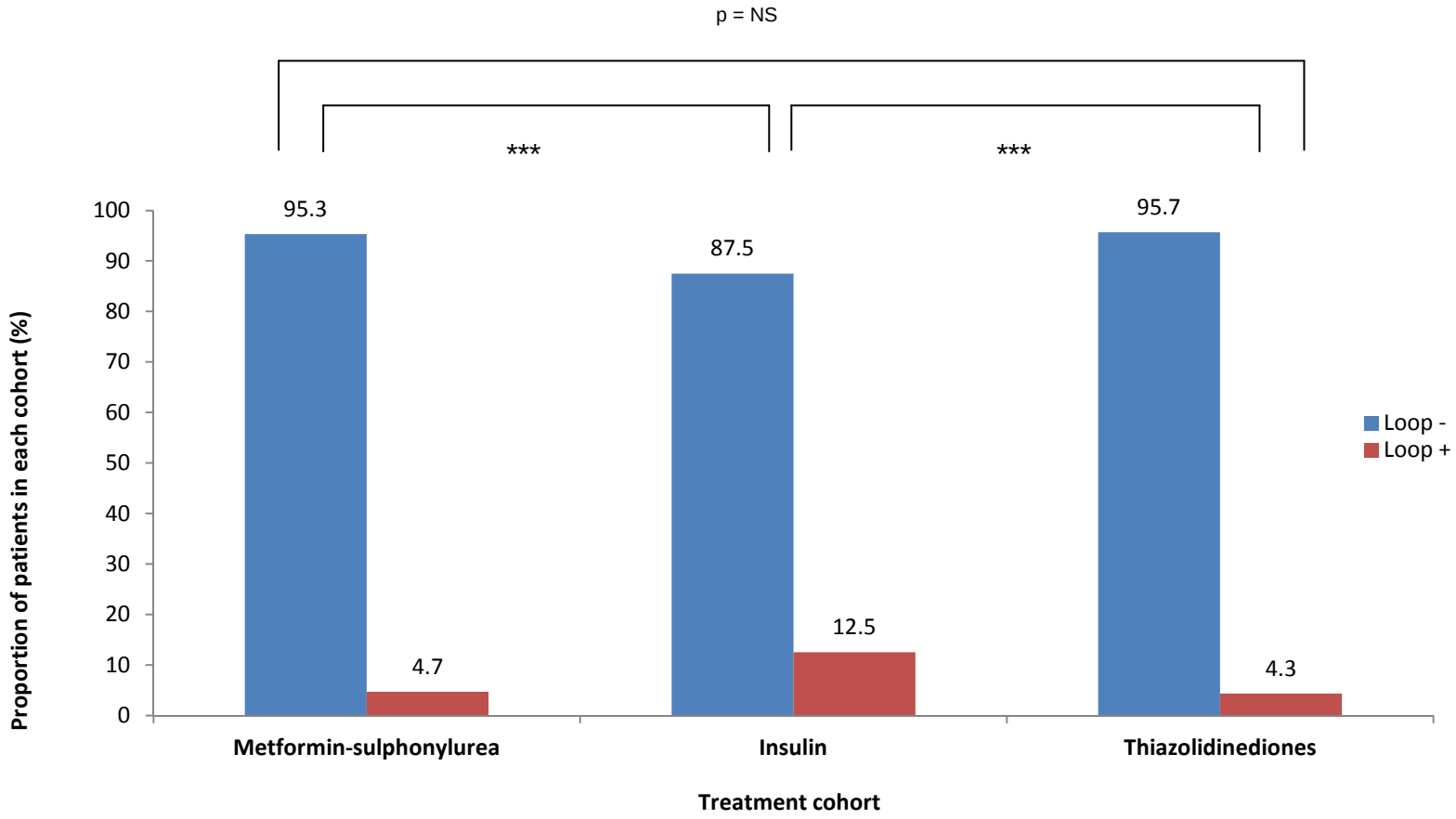
[a] *Chi square test for the overall difference between metformin-sulphonlylurea, insulin and thiazolidinedione cohorts*
[b] *Chi Square = 115.327, df = 2*
[c] *Chi Square = 59.101, df = 2*
[d] *Chi Square = 55.860, df = 2*

Table 3.16 - Post-hoc analysis: Chi square tests for the association between frequency of prescription of index loop diuretics within one year and inclusion into the metformin-sulphonylurea, insulin and thiazolidinedione cohorts for at least three months.

Gender subgroup	*Metformin-sulphonylurea cohort vs insulin cohort*			*Metformin-sulphonylurea cohort vs thiazolidinedione cohort*			*Insulin cohort vs thiazolidinedione cohort*		
	Chi square	*df*	*p*	*Chi square*	*df*	*p*	*Chi square*	*df*	*p*
Males and females	82.337	1	< 0.001	0.470	1	0.493	79.790	1	< 0.001
Males	33.920	1	< 0.001	3.510	1	0.061	49.323	1	< 0.001
Females	47.573	1	< 0.001	1.045	1	0.307	29.009	1	< 0.001

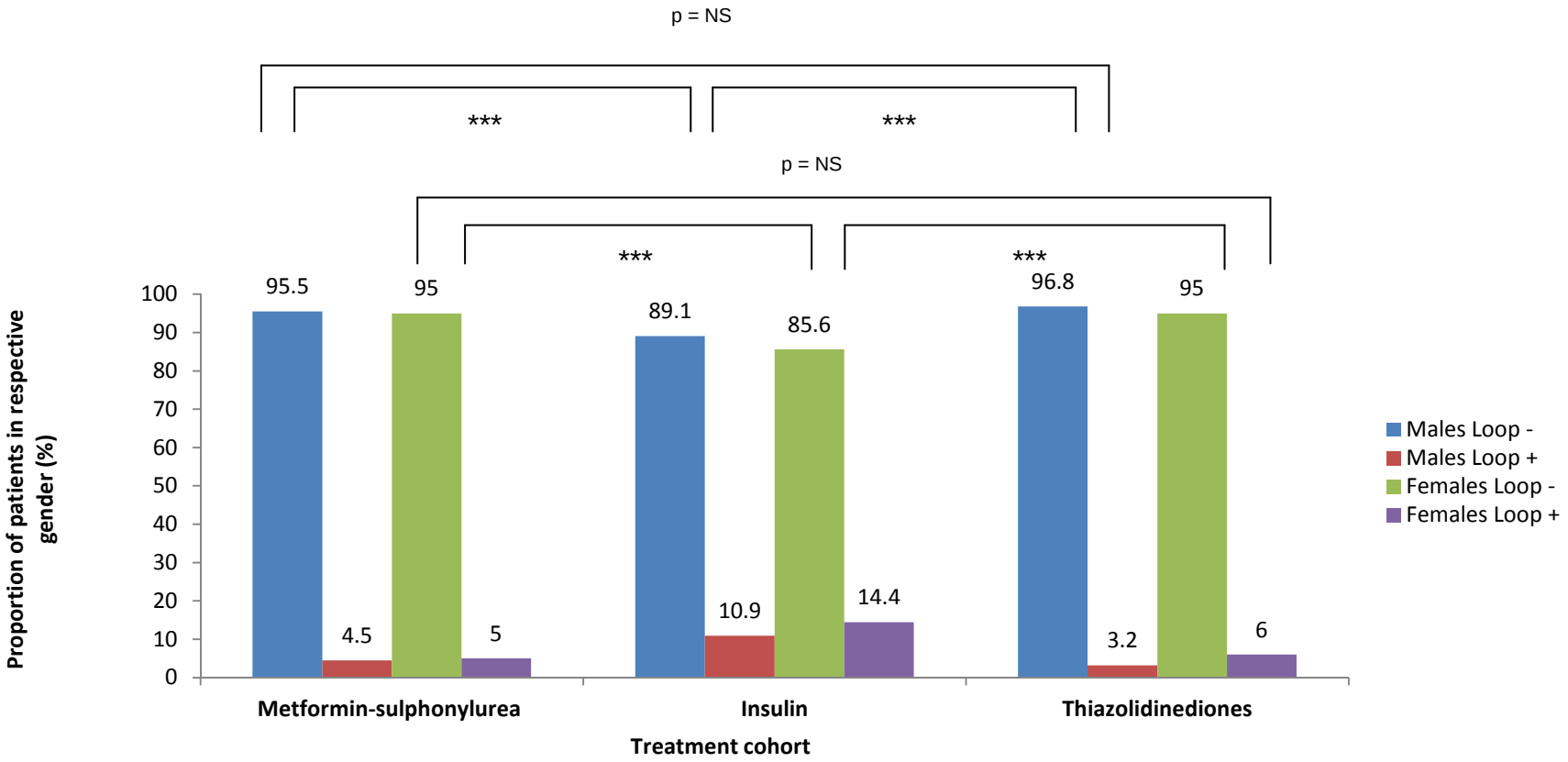
Tests of the three a priori hypotheses were conducted using Bonferroni adjusted alpha levels of 0.0167 per test (0.05/3).

Figure 3.5 - Relative proportions (%) of index loop diuretic prescription within one year of inclusion into each cohort.



*loop -, index loop diuretic-free; loop +, index loop diuretic-treated; *** p < 0.001; NS, no statistical difference; p< 0.001 for the overall difference in loop diuretic treatment counts across the three treatment cohorts; the three pairs of post-hoc tests were Bonferroni corrected.(statistical significance defined by a p value < 0.0167)*

Figure 3.6 - Relative proportions (%) of index loop diuretic prescription within one year of inclusion into each cohort, stratified by gender.



*loop -, index loop diuretic-free; loop +, index loop diuretic-treated; *** p < 0.001; NS, no statistical difference; p< 0.001 for the overall difference in loop diuretic treatment counts across the three treatment cohorts; the three pairs of post-hoc tests for each gender were Bonferroni corrected (statistical significance defined by a p value < 0.0167)*

Further investigating the increased risks associated with each treatment cohort, (unadjusted) odds ratios (OR) and relative risks (RR) (with 95% CI) were derived for each pairwise comparison. As outlined in table 3.17 below, the risk of requiring an index loop diuretic within one year of exposure to insulin is almost three times that of patients treated with thiazolidinediones. The risk is higher in insulin-treated males (3.4 fold) compared with insulin-treated female patients (2.4 fold).

Table 3.17 - Unadjusted relative risk of index loop diuretic prescription after exposure to index insulin therapy vs thiazolidinedione therapy

Gender status	*Unadjusted relative risk of index loop diuretic prescription after exposure to insulin (vs thiazolidinedione therapy)*	*95% confidence intervals*	
		Lower	*Upper*
Males and females	2.91	2.28	3.72
Males	3.44	2.38	4.97
Females	2.40	1.73	3.34

Insulin-treated patients have a 2.7 times higher risk of progressing to index loop diuretic prescription within one year compared to patients on metformin-sulphonylurea combination therapy [RR 2.66 (95% CI 2.13, 3.30)]. Similar results were obtained when stratifying by gender (table 3.18).

Table 3.18 - Unadjusted relative risk of index loop diuretic prescription after exposure to index insulin therapy vs metformin-sulphonylurea therapy

Gender status	*Unadjusted relative risk of index loop diuretic prescription after exposure to insulin (vs metformin-sulphonylurea therapy)*	*95% confidence intervals*	
		Lower	*Upper*
Males and females	2.66	2.13	3.30
Males	2.40	1.78	3.26
Females	2.91	2.12	4.00

As expected, given the non-significant differences in rates of index loop diuretic prescription between patients assigned a thiazolidinedione and those on metformin-sulphonylurea combination therapy, 95% CI for RR spanned unity, as outlined in table 3.19 below.

Table 3.19 Unadjusted relative risk of index loop diuretic prescription after exposure to index thiazolidinedione therapy (vs metformin-sulphonylurea therapy).

Gender status	*Unadjusted relative risk of index loop diuretic prescription following exposure to thiazolidinediones (vs metformin-sulphonylurea therapy)*	*95% confidence intervals*	
		Lower	*Upper*
Males and females	0.91	0.70	1.19
Males	0.70	0.48	1.02
Females	1.21	0.84	1.75

OR values for each of the three pairwise comparisons between thiazolidinediones, metformin-sulphonylurea combination therapy and insulin therapy are outlined in appendix I (appendix tables III.1 to III.3)

3.10.5 Kaplan-Meier survival curves for index loop diuretic therapy

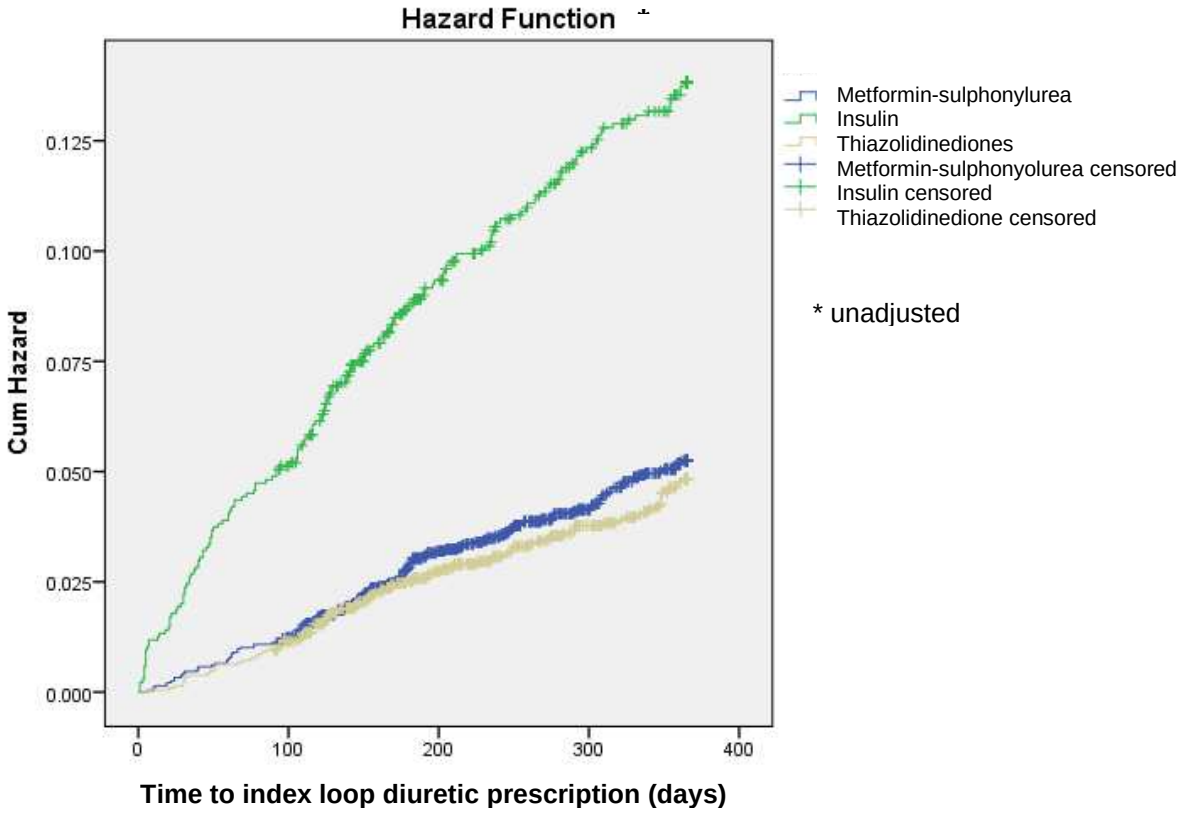
(Unadjusted) Kaplan-Meier survival curves were constructed to compare time to index loop diuretic prescription between the three treatment cohorts. Index insulin prescription was likely to be complicated by an index loop diuretic prescription at a significantly earlier stage than either of the other cohorts, as outlined in table 3.20 and figure 3.7 below. There were no significant difference in loop diuretic-free

survival rates between metformin-sulphonylurea combination and thiazolidinedione cohorts.

Table 3.20 - Survival (Kaplan-Meier) analysis comparing time to index loop diuretic prescription (censored at one year) after index metformin-sulphonylurea combination, insulin or thiazolidinedione therapy

Treatment cohort	*Log-rank (Mantel-Cox)*		
	Chi square	*df*	*p*
Metformin-sulphonylurea combination vs insulin vs thiazolidinediones	111.279	2	< 0.001
Metformin-sulphonylurea combination vs thiazolidinediones	0.420	1	0.517
Metformin-sulphonylurea combination vs insulin	79.035	1	< 0.001
Insulin vs thiazolidinediones	75.655	1	< 0.001

Figure 3.7 - Hazard curve comparing time to index loop diuretic prescription following index metformin-sulphonylurea combination, insulin and thiazolidinedione therapy



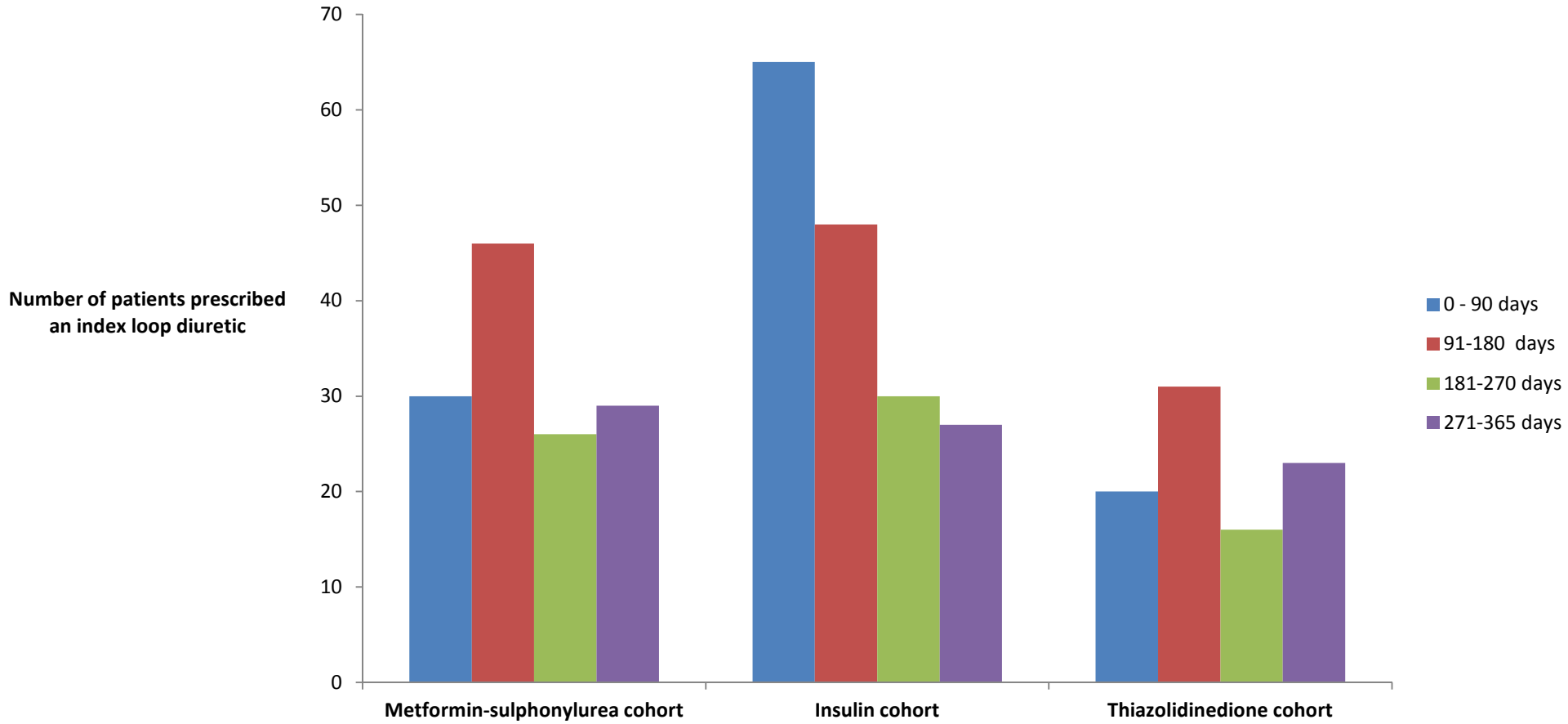
3.10.6 Timing of index loop diuretic prescription within a year after index metformin-sulphonylurea, insulin or thiazolidinedione therapy

Table 3.21 and figure 3.8 stratify the number of index loop diuretic prescription in three monthly intervals following index metformin-sulphonylurea, insulin and thiazolidinedione therapy. Index loop diuretic prescription is fairly evenly distributed throughout this period of observation for both metformin-sulphonylurea and thiazolidinedione-treated patients. This pattern contrasts with that exhibited for insulin-treated subjects, in whom index loop diuretic prescription becomes less likely over each progressive treatment quarter.

Table 3.21 - Index loop diuretic prescriptions stratified in three monthly intervals following index metformin-sulphonylurea combination, thiazolidinedione and thiazolidinedione therapy.

Treatment quarter	*Metformin-sulphonylurea cohort*	*Insulin cohort*	*Thiazolidinedione cohort*
0 - 90 days	30	65	20
91-180 days	46	48	31
181-270 days	26	30	16
271-365 days	29	27	23

Figure 3.8 - Number of patients prescibed an index loop diuretic stratified in three monthly intervals after index metformin-sulphonylurea, insulin and thiazolidinedione therapy



3.10.7 Occurrence of incident heart failure within one year of inclusion into each respective treatment cohort

Likewise, this study sought to investigate the rates of occurrence of incident HF within one year of inclusion into each respective cohort. This necessarily meant that patients with a background history of HF at inclusion were excluded, leaving 3476, 1815 and 2549 patients within the metformin-sulphonylurea, insulin and thiazolidinedione cohorts respectively. The relative proportions of patients defined as developing index HF within one year, based on SMR, echocardiography and loop diuretic data (as outlined in the methods section) is outlined in table 3.22 below.

Table 3.22 - Derivation of index heart failure (HF) data within one year of inclusion into the respective treatment cohort, based on data extraction definitions.

Index HF definition [1]	***Metformin-sulphonylurea cohort*** [4]	***Insulin cohort*** [4]	***Thiazolidinedione cohort*** [4]
Echo + loop data [2]			
Males and females	9	15	7
Males	8	6	5
Females	1	9	2
SMR [3] ***data***			
Males and females	40	50	21
Males	25	28	13
Females	15	22	8

[1] index HF definition, index heart failure developing within one year of inclusion into the respective treatment cohort; [2] echo + loop data, echocardiographic evidence of left ventricular systolic dysfunction and prescription of a loop diuretic within one year; [3] SMR, Scottish morbidity record; [4] number of patients captured based on each data extraction definition.

As outlined in tables 3.23 and 3.24, 1.1% of thiazolidinedione-treated patients developed incident HF within one year of prescription of their index rosiglitazoene or pioglitazone. This was not significantly different from patients on metformin-

sulphonylurea combination therapy (1.4%; $p = 0.288$), but significantly lower than for patients prescribed insulin (3.5%; $p < 0.001$). Stratifying by gender yielded similar results. These relative proportions are also summarised in figures 3.9 and 3.10.

Table 3.23 - Differences in frequency of occurrence of incident heart failure within one year after inclusion into the metformin-sulphonylurea, insulin and thiazolidinedione cohorts for at least three months.

Gender subgroup	*Metformin-sulphonylurea cohort* *N = 3476 (1933 males 1543 females)*				*Insulin cohort* *N = 1815 (893 males 922 females)*				*Thiazolidinedione cohort* *N = 2549 (1439 males 1110 females)*				*p*[a]
	Incident heart failure developed within one year		*Incident heart failure free within one year*		*Incident heart failure developed within one year*		*Incident heart failure free within one year*		*Incident heart failure developed within one year*		*Incident heart failure free within one year*		
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	
Males and females	49	1.4	3427	98.6	63	3.5	1752	96.5	28	1.1	2521	98.9	< 0.001[b]
Males	33	1.7	1900	98.3	32	3.6	861	96.4	18	1.3	1421	98.7	< 0.001[c]
Females	16	1.0	1527	99.0	31	3.4	891	96.6	10	0.9	1100	99.1	< 0.001[d]

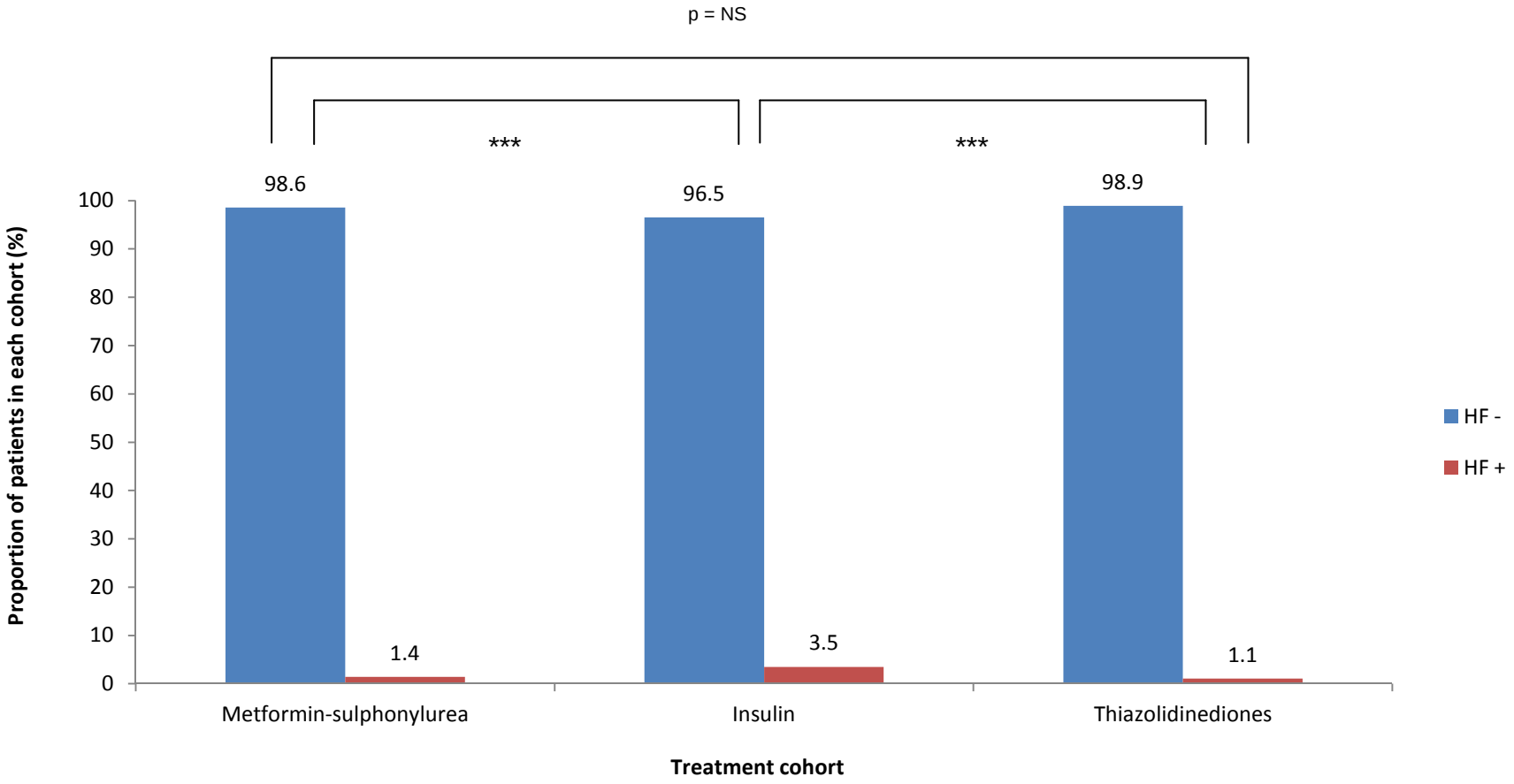
[a] *Chi square test for the overall difference between metformin-sulphonlylurea, insulin and thiazolidinedione cohorts*
[b] *Chi Square = 39.062, df = 2*
[c] *Chi Square = 16.769, df = 2*
[d] *Chi Square = 24.824, df = 2*

Table 3.24 - Post-hoc analysis: Chi square tests for the association between frequency of occurrence of incident heart failure within one year and inclusion into the metformin-sulphonylurea, insulin and thiazolidinedione cohorts for at least three months.

Gender subgroup	*Metformin-sulphonylurea cohort vs insulin cohort*			*Metformin-sulphonylurea cohort vs thiazolidinedione cohort*			*Insulin cohort vs thiazolidinedione cohort*		
	Chi square	***df***	***p***	***Chi square***	***df***	***p***	***Chi square***	***df***	***p***
Males and females	24.454	1	< 0.001	1.129	1	0.288	29.229	1	< 0.001
Males	9.569	1	0.002	1.153	1	0.283	14.290	1	< 0.001
Females	16.685	1	< 0.001	0.123	1	0.726	15.434	1	< 0.001

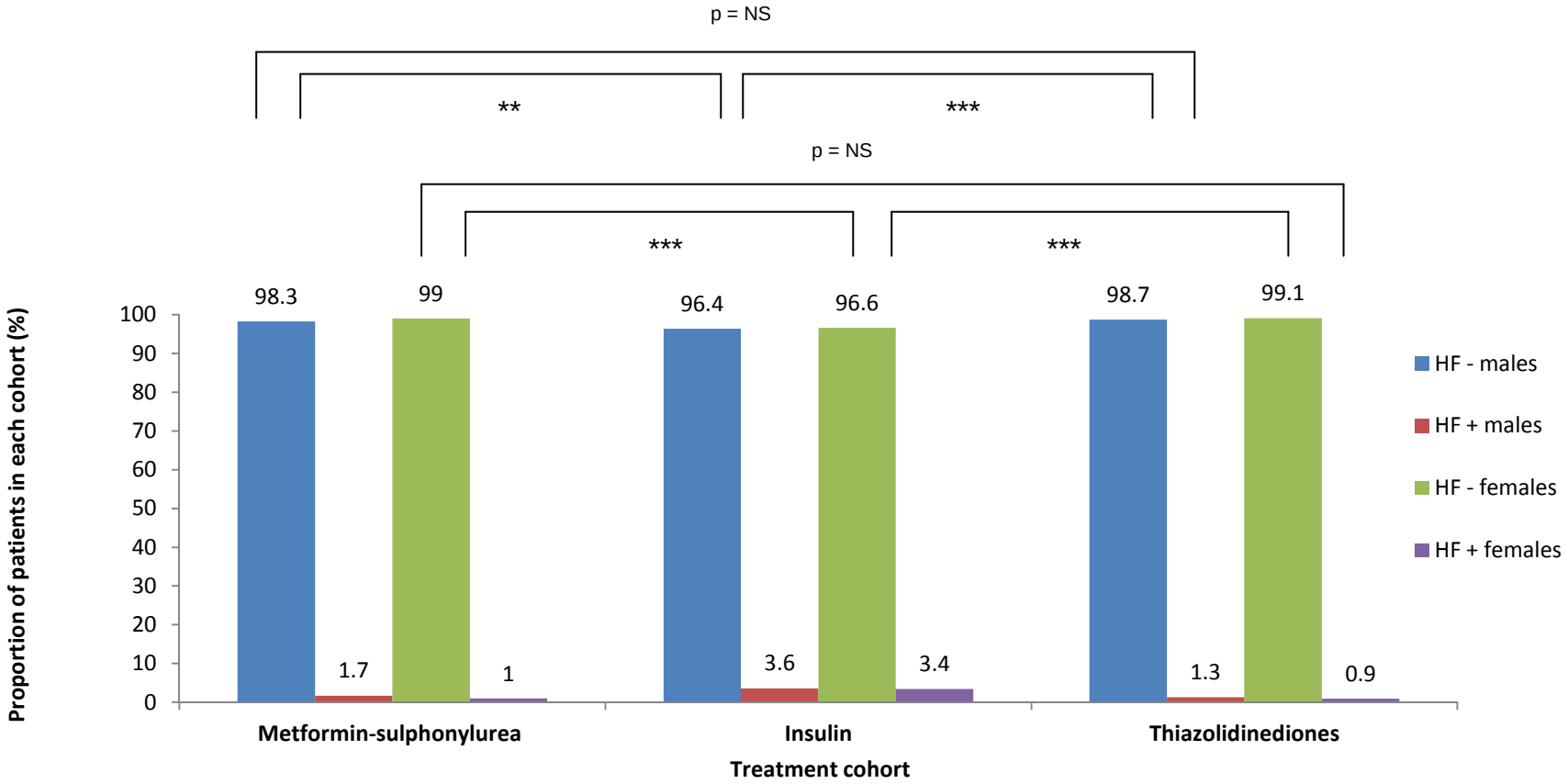
Tests of the three a priori hypotheses were conducted using Bonferroni adjusted alpha levels of 0.0167 per test (0.05/3).

Figure 3.9 - Relative proportions (%) of occurrence of incident heart failure (HF) within one year of inclusion into each cohort.



*p< 0.001 for the overall difference in loop diuretic treatment counts across the three treatment cohorts; HF -, incident heart failure free; HF +, developed incident heart failure within one year; *** p < 0.001; NS, no statistical differencethe three pairs of post-hoc tests were Bonferroni corrected.(statistical significance defined by a p value < 0.0167)*

Figure 3.10 - Relative proportions (%) of development of incident heart failure (HF) within one year of inclusion into each cohort, stratified by gender.



*HF -, incident heart failure free; HF +, developed incident heart failure within one year; *** p < 0.001; ** p < 0.01; NS, no statistical difference; p< 0.001 for the overall difference in loop diuretic treatment counts across the three treatment cohorts; the three pairs of post-hoc tests for each gender were Bonferroni corrected (statistical significance defined by a p value < 0.0167)*

Similarly, (unadjusted) RR ratios were derived for incident HF for each cohort pairwise comparison (tables 3.25 to 3.27). Thus, patients exposed to insulin are at a three fold risk of developing this adverse event compared with their thiazolidinedione-treated counterparts [RR 3.16 (95% CI 2.03, 3.72)] (table 3.25). This risk is higher for female insulin-treated patients [RR 3.73 (95% CI 1.84, 7.57)], albeit characterised by wider 95% CI. The latter probably arose on account of a relatively small number of female patients developing HF on subgroup analysis.

Table 3.25 - Unadjusted relative risk of occurrence of incident heart failure after exposure to index insulin therapy (vs thiazolidinedione therapy).

Gender status	*Unadjusted relative risk of incident heart failure after exposure to insulin (vs thiazolidinedione therapy)*	*95% confidence intervals*	
		Lower	*Upper*
Males and females	3.16	2.03	4.91
Males	2.87	1.62	5.07
Females	3.73	1.84	7.57

Similarly insulin therapy carries a 2.5 fold risk of progression to incident HF compared with metformin-sulphonylurea combination therapy [RR 2.46 (95% CI 1.70, 3.56)] (table 3.26). Female insulin-treated patients are more likely to develop this adverse event [RR 3.24 (95% CI 1.78, 5.90)].

Table 3.26 - Unadjusted relative risk of occurrence of incident heart failure after exposure to index insulin therapy (vs metformin-sulphonylurea therapy).

Gender status	*Unadjusted relative risk of incident heart failure following exposure to insulin (vs metformin-sulphonylurea therapy)*	*95% confidence intervals*	
		Lower	*Upper*
Males and females	2.46	1.70	3.56
Males	2.10	1.30	3.39
Females	3.24	1.78	5.90

Unadjusted RR values for exposure to thiazolidinediones vs metformin-sulphonylurea combination therapy were characterised by 95% CI which span unity (table 3.27), in keeping with the non-significant associations described earlier.

Table 3.27 - Unadjusted relative risk of occurrence of incident heart failure after exposure to index thiazolidinedione therapy (vs metformin-sulphonylurea therapy).

Gender status	*Unadjusted odds ratio of incident heart failure following exposure to thiazolidinediones (vs metformin-sulphonylurea therapy)*	*95% confidence intervals*	
		Lower	*Upper*
Males and females	0.779	0.491	1.236
Males	0.733	0.414	1.296
Females	0.869	0.396	1.907

Derived ORs for each of the three pair-wise comparisons between treatment cohorts are summarised in appendix tables III.4 to III.6.

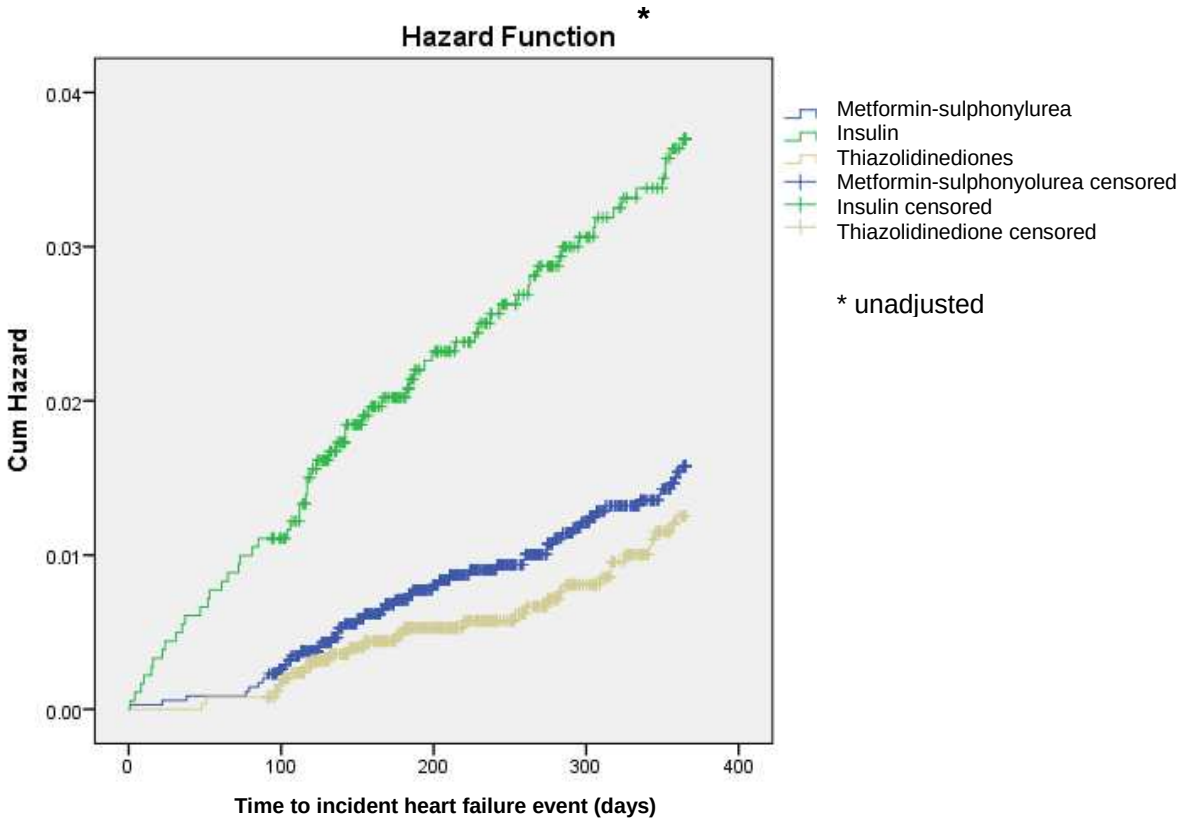
3.10.8 Kaplan-Meier survival curves for incident heart failure

Pairwise log-rank (Mantel-Cox) p values comparing time to index loop diuretic prescription between the three treatment cohorts were consistent with the above results, confirming significantly earlier progression to incident HF for insulin-treated patients, and comparable HF free survival times for thiazolidinedione and metformin-sulphonylurea combination cohorts (table 3.28, figure 3.11).

Table 3.28 - Survival (Kaplan Meier) analysis comparing time to incident heart failure (censored at one year) after index metformin-sulphonylurea combination, insulin or thiazolidinedione therapy

Treatment cohort	*Log-rank (Mantel-Cox)*		
	Chi square	*df*	*p*
Metformin-sulphonylurea combination vs insulin vs thiazolidinediones	35.990	2	< 0.001
Metformin-sulphonylurea combination vs thiazolidinediones	1.089	1	0.297
Metformin-sulphonylurea combination vs insulin	22.494	1	< 0.001
Insulin vs thiazolidinediones	27.015	1	< 0.001

Figure 3.11 - Hazard curves comparing time to incident heart failure following index metformin-sulphonylurea, insulin or thiazolidinedione therapy



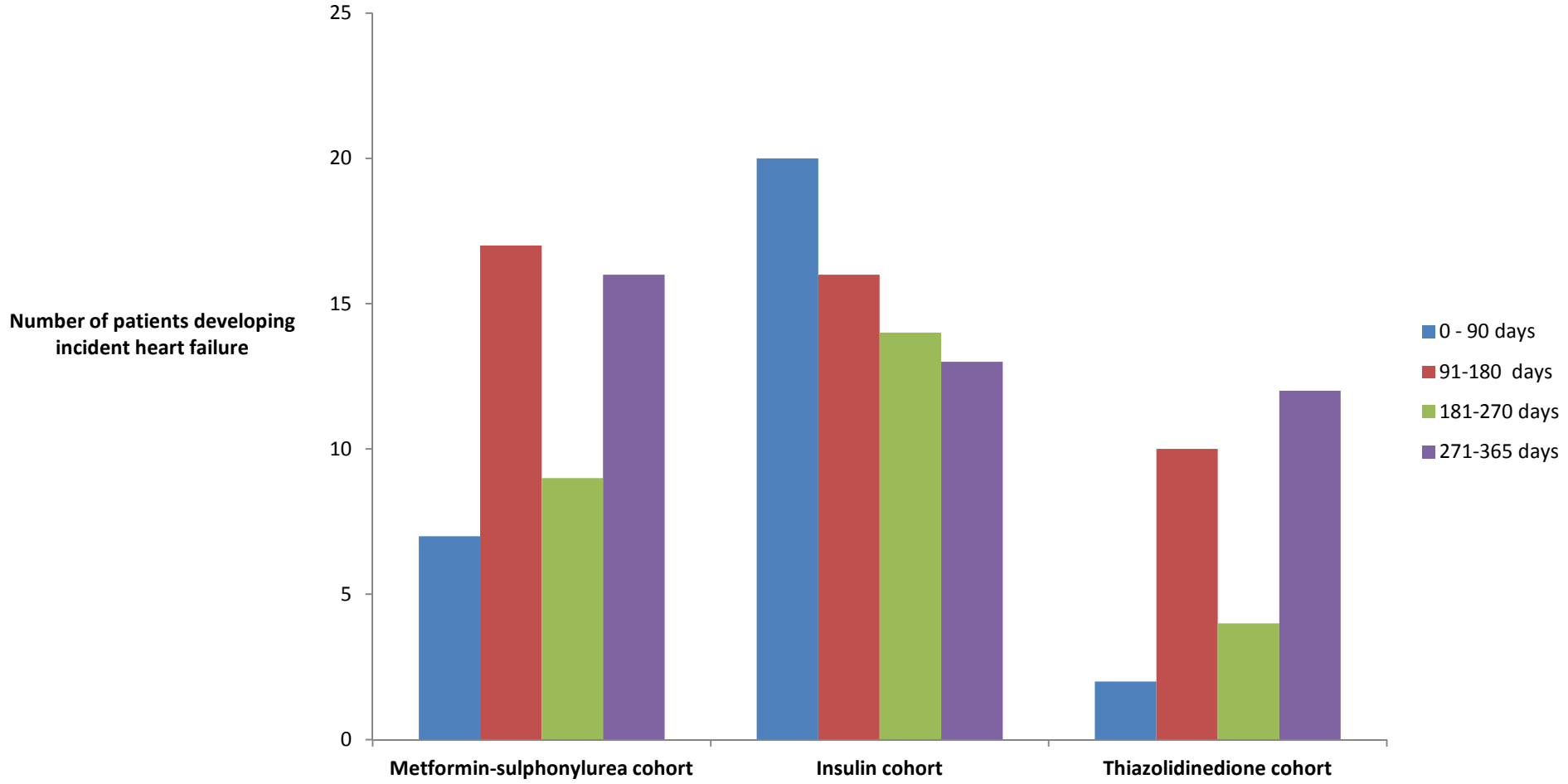
3.10.9 Timing of incident heart failure events within a year after index metformin-sulphonylurea, insulin or thiazolidinedione therapy

Timing of incident HF events largely mirrors that for index loop diuretic prescription, with occurrences of new-onset HF becoming progressively less likely at each successive three month interval following index insulin prescription. Incident HF events were more or less randomly distributed following index metformin-sulphonylurea and thiazolidinedione therapy, as outlined in table 3.29 and figure 3.12 below.

Table 3.29 - Incident heart failure events stratified in three monthly intervals following index metformin-sulphonylurea combination, thiazolidinedione and thiazolidinedione therapy.

Treatment quarter	*Metformin-sulphonylurea cohort*	*Insulin cohort*	*Thiazolidinedione cohort*
0 - 90 days	7	20	2
91-180 days	17	16	10
181-270 days	9	14	4
271-365 days	16	13	12

Figure 3.12 - Number of incident heart failure events occurring at three monthly intervals after index metformin-sulphonylurea, insulin and thiazolidinedione therapy



3.11 Baseline characteristics

3.11.1 Age, diabetes duration and duration of follow-up

Table 3.30 outlines mean (SD) values for age, diabetes duration and study duration for patients without background loop diuretic therapy. Thiazolidinedione-treated patients tended to be younger than their metformin-sulphonylurea [63.23 (9.77) vs 64.96 (10.53) years; $p < 0.001$] and insulin-treated [63.23 (9.77) vs 64.92 (10.13) years; $p < 0.001$] counterparts. As perhaps expected for a second/third line glucose lowering agent such as a thiazolidinedione, patients prescribed the latter drugs tended to have been diagnosed with diabetes at an earlier stage than patients on metformin-sulphonylurea combination therapy [6.86 (4.90) vs 5.31 (4.74) years; $p < 0.001$], although not as long as for insulin-treated subjects [6.86 (4.90) vs 8.70 (6.02) years; $p < 0.001$]. Thiazolidinedione-treated patients had the shortest follow-up observation period [3.02 (2.16) years], possibly reflecting tendency to drug withdrawal on developing/suspicion of developing adverse effects to these drugs. This duration of follow-up was significantly shorter than for insulin [6.22 (4.10) years] and metformin-sulphonylurea [3.53 (3.02) years] treated patients ($p < 0.001$ for either treatment cohort vs thiazolidinediones).

Table 3.31 outlines the mean (SD) values for age, diabetes duration and years of follow-up for each treatment cohort, stratified by index loop diuretic status and gender. Thiazolidinedione-treated patients requiring an index loop diuretic within one year of inclusion into the cohort were older [67.98 (10.02) vs 63.02 (9.70) years; $p < 0.001$], and had been diagnosed with diabetes at a significant earlier stage [8.44

(5.61) vs 6.79 (4.85) years; p = 0.003] than their index loop diuretic-free counterparts. Index loop diuretic-treated TZD patients were also characterised by a tendency for a shorter observation period of follow-up (albeit not statistically significant) [2.60 (1.92) vs 3.04 (2.17) years; p = 0.056], once again, possibly reflecting a tendency to discontinue thiazolidinedione therapy, or shorter survival once there is clinical evidence of fluid overload. A similar analysis of incident HF events occurring within one year of inclusion into each of the three treatment cohorts (HF +) yielded largely similar results, albeit with differences in duration of follow-up [1.50 (1.65) (HF +) vs 2.98 (2.12) (HF –) years) reaching statistical significance (p < 0.001), in contrast to those for diabetes duration [8.15 (4.88) (HF +) vs 6.93 (5.01) (HF –) years; p = 0.136) (data not shown in table format).

Likewise, MFSU patients treated with an index loop diuretic after inclusion into the cohort were older [69.21 (9.81) vs 64.75 (10.52) years; p < 0.001], and had a longer duration of diabetes [6.56 (5.50) vs 5.24 (4.69) years; p = 0.005] compared with their index loop diuretic-free counterparts. The duration of follow-up of these patients was largely similar [3.46 (3.16) vs 3.53 (3.01) years; p = 0.669] (table 3.31), an observation that is perhaps not entirely surprising given the lack of a known association between metformin-sulphonylurea combination therapy and fluid overload. Similar trends were reported for incident HF events, with differences being more pronounced, and reaching statistical significance with respect to duration of follow-up [1.99 (2.24) (HF +) vs 3.49 (2.96) (HF -) years; p < 0.001) (data not shown in table format). This suggests that once HF sets in, patients are either (i) characterised by a shorter survival, or (ii) more likely to be switched to more intensive glucose lowering therapy (such as insulin).

Insulin-treated patients requiring an index loop diuretic were older [68.30 (9.24) vs 64.44 (10.16) years; $p < 0.001$] and likely to be observed for a significantly shorter period after inclusion into their respective cohort [5.18 (3.81) vs 6.37 (4.11) years; $p < 0.001$] (table 3.31). Whilst discontinuation of insulin therapy is unlikely at such a late stage of the disease, a shorter observation period could reflect higher mortality rates for index loop diuretic-treated patients in this cohort. Diabetes duration was similar in either insulin subgroup [9.12 (6.61) vs 8.65 (5.93) years; $p = 0.420$] (table 3.31). Similar results were reported for incident HF events (data not shown in table format), with particularly pronounced, statistically significant, differences in duration (years) of follow-up [3.30 (3.13) (HF +) vs 5.99 (4.06) (HF -) years; $p < 0.001$].

Table 3.30 - Comparison of mean (SD) values for baseline age, diabetes duration and study duration for patients treated with metformin-sulphonylurea combination, insulin and thiazolidinedione therapy for at least three months, and having no background loop diuretic therapy at inclusion into their respective cohort.

	Metformin-sulphonylourea cohort ***N = 2785***	***Insulin cohort*** ***N = 1361***	***Thiazolidinedione (TZD) cohort*** ***N = 2097***	***p value for the difference across the three cohorts*** [a]	***p value for metformin-sulphonylurea cohort vs insulin cohort*** [b]	***p value for metformin-sulphonylurea cohort vs TZD cohort*** [b]	***p value for insulin cohort vs TZD cohort*** [b]
Age (years)	64.96 (10.53)	64.92 (10.13)	63.23 (9.77)	< 0.001	0.993[c]	< 0.001[c]	< 0.001[c]
Diabetes duration (years)	5.31 (4.74)	8.70 (6.02)	6.86 (4.90)	< 0.001[d]	< 0.001[c, d]	< 0.001[c, d]	< 0.001[c, d]
Study duration (years)	3.53 (3.02)	6.22 (4.10)	3.02 (2.16)	< 0.001[d]	< 0.001[c, d]	< 0.001[c, d]	< 0.001[c, d]

[a] two-tailed p value [One-way analysis of variance (ANOVA)]; [b] two-tailed p value (pair-wise post-hoc analysis). Tests of the three a priori hypotheses were conducted using the Games-Howell test [c]; [d] differences calculated on square root transformed data

Table 3.31 - Comparison of mean (SD) values for age, duration of diabetes and years of follow-up between individuals requiring treatment with loop diuretics and those remaining loop diuretic-free within one year after exposure to metformin-sulphonylurea combination, insulin or thiazolidinedione therapy for a minimum of three months, and having no background loop diuretic therapy at inclusion into their respective cohort.

	Metformin-Sulphonylurea cohort			***Insulin cohort***			***Thiazolidinedione cohort***		
	Loop diuretic -treated	***Loop diuretic -free***	***p[a]***	***Loop diuretic -treated***	***Loop diuretic -free***	***p[a]***	***Loop diuretic -treated***	***Loop diuretic -free***	***p[a]***
	N = 131	***N = 2654***		***N = 170***	***N = 1191***		***N = 90***	***N = 2007***	
Age (years)	69.21 (9.81)	64.75 (10.52)	< 0.001	68.30 (9.24)	64.44 (10.16)	< 0.001	67.98 (10.02)	63.02 (9.70)	< 0.001
Diabetes duration (years)	6.56 (5.50)	5.24 (4.69)	0.005 [b]	9.12 (6.61)	8.65 (5.93)	0.420 [b]	8.44 (5.61)	6.79 (4.85)	0.003 [b]
Study duration (years)	3.46 (3.16)	3.53 (3.01)	0.669 [b]	5.18 (3.81)	6.37 (4.11)	< 0.001	2.60 (1.92)	3.04 (2.17)	0.056 [b]

[a] two-tailed p vslue for the difference between loop diuretic- treated and loop diuretic- free patients [One-way analysis of variance (ANOVA)]; [b] differences calculated on square root transformed data

3.11.2 Past medical history

Analyzing data pertaining to these patients' past medical history, 317 (15.1%) of patients with no background loop diuretic therapy were prescribed an index thiazolidinedione on a background of known coronary artery disease or peripheral arterial disease or stroke. This is considerably lower than for metformin-sulphonylurea- [549 (19.7%); $p < 0.001$] and insulin- [363 (26.7%); $p < 0.001$] treated patients (table 3.32). Analyzing these macrovascular complications separately, these *post-hoc* pairwise comparisons reached statistical significance only for coronary artery disease and stroke. There was no significant difference in the frequency of background peripheral artery disease between metformin-sulphonylurea and thiazolidinedione-treated patients, although the latter were significantly less likely to suffer from background PAD at index TZD prescription compared with their insulin-treated counterparts [43 (2.1%) vs 67 (4.9%); $p < 0.001$] (table 3.32).

Analyzing for individuals who had never been prescribed a loop diuretic before inclusion into their respective cohort, there were no significant differences in the frequencies of background HF between thiazolidinedione and metformin-sulphonylurea-treated patients [18 (0.9%) (TZD) vs 31 (1.1%) (MFSU); $p = 0.377$] (table 3.32). Lower background rates for HF at an early stage of T2DM (when metformin-sulphonylurea combination therapy is likely to be prescribed) are likely to be offset by lower background rates of HF among patients prescribed thiazolidinediones in accordance with established treatment guidelines. Insulin is statistically more likely to be prescribed in patients known to suffer from HF compared with thiazolidinediones [69 (5.1%) vs 18 (0.9%); $p < 0.001$], again

probably reflecting (i) consensus guided prescribing practices (thiazolidinediones are contraindicated in patients with HF) and (ii) the fact that insulin requiring patients are more prone to coronary artery disease with complicating HF given the more advanced stage of their disease.

Interestingly, excluding patients with background loop diuretic therapy, HF rates following inclusion into the respective treatment cohorts were lowest for thiazolidinediones [64 (3.1%)], significantly less than for metformin-sulphonylurea [162 (5.8%); $p < 0.001$] or insulin [209 (15.4%); $p < 0.001$] (table 3.32), although this comparison must be interpreted with caution, as (i) these patients were followed up for a significantly shorter period than their metformin-sulphonylurea and insulin-treated counterparts, and (ii) background HF rates were significantly lower for thiazolidinedione-treated patients compared with insulin-treated ones. Similar observations apply to post-treatment coronary artery disease, stroke and peripheral artery disease, or their composite.

Patients requiring an index loop diuretic within one year after index thiazolidinedione prescription were more likely to suffer from background coronary artery disease [18 (20.0%) vs 225 (11.2%); $p = 0.011$], peripheral artery disease [6 (6.7%) vs 37 (1.8%); $p = 0.009$] or the composite of macrovasular disease [27 (30.2%) vs 290 (14.4%); $p < 0.001$] (table 3.33). Although background stroke rates were higher among patients requiring an index loop diuretic after TZD prescription [4 (4.6%) vs 53 (2.6%)], these differences did not reach statistical significance, possibly as a result of the relatively smaller number of patients with this disease category at TZD prescription. Similar differences, namely higher background rates

of coronary artery disease, peripheral artery disease and the composite of macrovascular disease were observed for index loop diuretic requiring metformin-sulphonylurea-treated patients (table 3.33). Analyzing for incident HF events, both thiazolidinedione and metformin-sulphonylurea combination therapy patients diagnosed with new-onset HF within one year of inclusion into either cohort were likewise characterised by significantly higher rates of background coronary artery disease [11 (39.3%) vs 337 (13.4%), $p = 0.001$ for thiazolidinedione-treated patients; 22 (44.9%) vs 540 (15.8%), $p < 0.001$ for MFSU-treated patients]. Higher background stroke rates among HF prone TZD- and MFSU-treated patients did not reach statistical significance – however, the number of incident HF events was particularly low for either cohort [3 (MFSU) and 2 (TZD)], rendering statistical interpretation somewhat dubious (data not shown in table format).

Insulin-treated patients requiring requiring an index loop diuretic within one year after inclusion into this glucose lowering treatment category were more likely to have suffered from coronary artery disease [63 (37.1%) vs 214 (18.0%); $p < 0.001$] or the composite of macrovascular disease [74 (43.5%) vs 289 (24.3%); $p < 0.001$] at baseline. (table 3.33). Higher rates of index loop diuretic prescription among insulin-treated patients with a history of stroke or peripheral artery disease did not reach statistical significance. Similar results were replicated for incident HF events in this treatment cohort (data not shown).

Perhaps not surprisingly, index loop diuretic prescription was commoner among metformin-sulphonylurea or insulin-treated patients prescribed these drugs on a background of HF [6 (4.6%) vs 25 (0.9%); $p = 0.003$ (metformin-sulphonylurea); 32

(18.8%) vs 37 (3.1%); $p < 0.001$ (insulin)]. Such a difference, although reported for thiazolidinediones [2 (2.2%) vs 16 (0.8%)] did not reach statistical difference, probably because of lower rates of background HF for this cohort (table 3.33).

Table 3.32 - Comparison of the relative frequency [n (%)] of background and post-treatment macrovascular disease and heart failure among patients treated with metformin-sulphonylurea combination, insulin or thiazolidinedione therapy for a minimum of three months, and having no background loop diuretic therapy at inclusion into their respective cohort.

	Metformin-sulphonylourea cohort N = 2785	*Insulin cohort N = 1361*	*Thiazolidinedione (TZD) cohort N = 2097*	*p value for the difference across the three cohorts* [a]	*p value for metformin-sulphonylurea cohort vs insulin cohort* [b]	*p value for metformin-sulphonylurea cohort vs TZD cohort* [b]	*p value for insulin cohort vs TZD cohort* [b]
Background CAD	397 (14.3)	277 (20.4)	243 (11.6)	< 0.001	< 0.001	0.006	< 0.001
Post-treatment CAD	358 (12.9)	410 (30.1)	170 (8.1)	< 0.001	< 0.001	< 0.001	< 0.001
Background stroke	152 (5.5)	85 (6.2)	57 (2.7)	< 0.001	0.305	< 0.001	< 0.001
Post-treatment stroke	127 (4.6)	147 (10.8)	39 (1.9)	< 0.001	< 0.001	< 0.001	< 0.001
Background PAD	76 (2.7)	67 (4.9)	43 (2.1)	< 0.001	< 0.001	0.128	< 0.001
Post-treatment PAD	93 (3.3)	137 (10.1)	27 (1.3)	< 0.001	< 0.001	< 0.001	< 0.001
Background macrovasc disease	549 (19.7)	363 (26.7)	317 (15.1)	< 0.001	< 0.001	< 0.001	< 0.001
Post-treatment macrovasc disease	490 (17.6)	534 (39.2)	217 (10.3)	< 0.001	< 0.001	< 0.001	< 0.001

CAD, coronary artery disease; macrovasc disease, composite of macrovascular disease comprising a history of known coronary artery disease or peripheral arterial disease or stroke; PAD, peripheral arterial disease; [a] *two-tailed p value (Chi Square test),* [b] *two-tailed p value (Chi Square test). Tests of the three a priori hypotheses were conducted using Bonferroni adjusted alpha lelves of 0.0167 per test (0.05/3).*

	Metformin-sulphonylourea cohort *N = 2785*	*Insulin cohort* *N = 1361*	*Thiazolidinedione (TZD) cohort* *N = 2097*	*p value for the difference across the three cohorts* [a]	*p value for metformin-sulphonylurea cohort vs insulin cohort* [b]	*p value for metformin-sulphonylurea cohort vs TZD cohort* [b]	*p value for insulin cohort vs TZD cohort* [b]
Background HF	31 (1.1)	69 (5.1)	18 (0.9)	< 0.001	< 0.001	0.377	< 0.001
Post-treatment HF	162 (5.8)	209 (15.4)	64 (3.1)	< 0.001	< 0.001	< 0.001	< 0.001

HF, heart failure; [a] two-tailed p value (Chi Square test), [b] two-tailed p value (Chi Square test). Tests of the three a priori hypotheses were conducted using Bonferroni adjusted alpha levels of 0.0167 per test (0.05/3)

Table 3.33 - Comparison of the relative frequency of background and post-treatment macrovascular disease and heart failure between individuals requiring treatment with loop diuretics and those remaining loop diuretic-free within one year after exposure to metformin-sulphonylurea combination, insulin or thiazolidinedione therapy for a minimum of three months, and having no background loop diuretic therapy at inclusion into their respective cohort.

	Metformin-Sulphonylurea cohort			***Insulin cohort***			***Thiazolidinedione cohort***		
	Loop diuretic -treated	***Loop diuretic -free***	***p [a, b]***	***Loop diuretic -treated***	***Loop diuretic -free***	***p [a, b]***	***Loop diuretic -treated***	***Loop diuretic -free***	***p [a, b]***
	N = 131	***N = 2654***		***N = 170***	***N = 1191***		***N = 90***	***N = 2007***	
Background CAD	35 (26.7)	362 (13.6)	< 0.001 [a]	63 (37.1)	214 (18.0)	< 0.001 [a]	18 (20.0)	225 (11.2)	0.011 [a]
Post-treatment CAD	37 (28.2)	321 (12.1)	< 0.001 [a]	80 (47.1)	330 (27.7)	< 0.001 [a]	15 (16.7)	155 (7.7)	0.002 [a]
Background stroke	10 (7.6)	142 (5.4)	0.261 [a]	12 (7.1)	73 (6.1)	0.639 [a]	4 (4.4)	53 (2.6)	0.303 [a]
Post-treatment stroke	11 (8.4)	116 (4.4)	0.031 [a]	26 (15.3)	121 (10.2)	0.044 [a]	2 (2.2)	37 (1.8)	0.683 [b]
Background PAD	10 (7.6)	66 (2.5)	0.003 [b]	12 (7.1)	55 (4.6)	0.169 [a]	6 (6.7)	37 (1.8)	0.009 [b]
Post-treatment PAD	9 (6.9)	84 (3.2)	0.039 [b]	21 (12.4)	116 (9.7)	0.289 [a]	2 (2.2)	25 (1.2)	0.324 [b]

CAD, coronary artery disease; PAD, peripheral arterial disease; [a] two-tailed p value for the statistical difference between loop diuretic-treated and loop diuretic-free patients (Chi Square test); [b] two-tailed p value for the statistical difference between loop diuretic-treated and loop diuretic-free patients (Fisher's exact tes

	Metformin-Sulphonylurea cohort			***Insulin cohort***			***Thiazolidinedione cohort***		
	Loop diuretic -treated	***Loop diuretic -free***	***p [a, b]***	***Loop diuretic -treated***	***Loop diuretic -free***	***p [a, b]***	***Loop diuretic -treated***	***Loop diuretic -free***	***p [a, b]***
	N = 131	***N = 2654***		***N = 170***	***N = 1191***		***N = 90***	***N = 2007***	
Background macrovasc disease	47 (35.9)	502 (18.9)	< 0.001 [a]	74 (43.5)	289 (24.3)	< 0.001 [a]	27 (30.2)	290 (14.4)	< 0.001 [a]
Post-treatment macovasc disease	46 (35.1)	444 (16.7)	< 0.001 [a]	98 (57.6)	436 (36.6)	< 0.001 [a]	17 (18.9)	200 (10.0)	0.007 [a]
Background HF	6 (4.6)	25 (0.9)	0.003 [b]	32 (18.8)	37 (3.1)	< 0.001 [a]	2 (2.2)	16 (0.8)	0.179 [b]
Post-treatment HF	25 (19.1)	137 (5.2)	< 0.001 [a]	63 (37.1)	146 (12.3)	< 0.001 [a]	12 (13.3)	52 (2.6)	< 0.001 [b]

HF, heart failure; macrovasc, composite of macrovascular disease comprising a history of known coronary artery disease or peripheral artery disease or stroke; [a] two-tailed p value for the statistical difference between loop diuretic- treated and loop diuretic- free patients (Chi Square test); [b] two-tailed p value for the statistical difference between loop diuretic- treated and loop diuretic- free patients (Fisher's exact test)

3.11.3 Drug history

This study captured data pertaining to a wide range of drugs which could possibly, at least partly, explain an increased risk for fluid overload following index thiazolidinedione prescription. Thiazolidinedione-treated patients were more likely to be prescribed these oral glucose lowering agents on a background of peripheral vasodilators (3.2%), thiazide diuretics (35.8%), non-steroidal anti-inflammatory drugs (69.8%), angiotensin converting enzyme inhibitors (54.9%) and aldosterone receptor antagonists (14.9%), compared with their metformin-sulphonylurea or insulin-treated counterparts (table 3.34).

Thiazolidinedione-treated patients were additionally characterised by higher background rates of dihydropyridine calcium channel blockers (35.4%), diltiazem (6.5%), beta blockers (40.1%), nitrates (18.7%) and other anti-anginal drugs (2.2%) and lower background prescription of peripheral vasocilators (3.2%) compared with patients on insulin. Antecedent prescription of alpha adrenoceptor blocking drugs was commoner among thiazolidinedione prescribed patients (8.9%) compared with metformin-sulphonylurea-treated ones (table 3.34).

Searching for possible causes of fluid overload, this study compared frequencies of background drug therapy between patients requiring loop diuretic (LD+), and those remaining loop diuretic-free (LD-) after index thiazolidinedione therapy (table 3.35). The former patients were more likely to be treated with a nitrate [25 (27.8%) (LD+) vs 367 (18.3%) (LD-); $p = 0.024$], in keeping with higher rates of coronary artery disease among this category of thiazolidinedione-treated patients. Higher

background use of thiazides [41 (45.6%) (LD+) vs 710 (35.4%) (LD-)] was borderline statistically significant (p = 0.049).

Patients were more likely to require an index prescription of a loop diuretic after index metformin-sulphonylurea combination therapy if the latter was introduced against a background of peripheral vasodilators [12 (9.2%) (LD+) vs 118 (4.4%) (LD-); p = 0.013), dihydropyridine calcium channel blockers [55 (42.0%) (LD+) vs 853 (32.1%) (LD-); p = 0.019], diltiazem [19 (14.5%) (LD+) vs 170 (6.4%) (LD-); p < 0.001); beta blockers [64 (48.9%) (LD+) vs 982 (37.0%) (LD-); p = 0.006] or nitrates [52 (39.7%) (LD) vs 532 (20.0%) (LD-); p < 0.001] (table 3.35).

T2DM patients treated with insulin were more likely to require treatment with an index loop diuretic after their index insulin prescription if the latter was introduced on a background of thiazide diuretics [46 (27.1%) (LD+) vs 237 (19.9%) (LD-); p = 0.031], diltiazem [25 (14.7%) (LD+) vs 115 (9.7%) (LD-); p = 0.043], alpha adrenoceptor blocking drugs [22 (12.9%) (LD+) vs 75 (6.3) (LD-); p = 0.002], angiotensin II receptor antagonists [16 (9.4%) (LD+) vs 58 (4.9%) (LD-); p = 0.015] or nitrates [60 (35.3%) (LD+) vs 280 (23.5%) (LD-); p = 0.001] (table 3.35).

Likewise, a comparison of background drug prescription among individuals developing incident heart failure (HF+), and those remaining heart failure free (HF-) within one year of inclusion into each of the three treatment cohorts yielded provocative but preliminary results (data not shown), as interpretation was limited in by small numbers of patients being prescribed less commonly used drugs, especially in the context of a relatively infrequent adverse event of interest (HF). Thus, patients

developing incident HF within one year of their index thiazolidinedione prescription were more likely to have had their oral glucose lowering agent introduced against a background of verapamil [4 (14.3%) (HF+) vs 34 (1.3%) (HF-); p = 0.001], diltiazem [7 (25.0%) (HF+) vs 206 (8.2%) (HF-); p = 0.007], beta blockers [18 (64.3%) (HF+) vs 1084 (43.0%) (HF-); p = 0.024], and nitrates [16 (57.1%) (HF+) vs 548 (21.7%) (HF-); p < 0.001]. Analyzing for metformin-sulphonylurea combination therapy, patients were more likely to be diagnosed with new-onset HF if their glucose lowering therapy was prescribed while on potassium sparing diuretics/aldosterone antagonists [8 (16.3%) (HF+) vs 148 (4.3%) (HF-); p = 0.001], beta blockers [28 (57.1%) (HF+] vs 1402 (40.9%) (HF-); p = 0.022] or nitrates [25 (51.0%) (HF+) vs 831 (24.2%) (HF-); p <0 .001]. Patients whose insulin therapy was commenced while on potassium sparing diuretics/aldosterone antagonists [10 (15.9%) (HF+) vs 99 (5.7%) (HF-); p = 0.003], non-steroidal anti-inflammatory drugs [50 (79.4%) (HF+) vs 1148 (65.5%) (HF-); p = 0.023], dihydropyridine calcium channel blockers [34 (54.0%) (HF+) vs 673 (38.4%); p = 0.013], diltiazem [18 (28.6%) (HF+) vs 213 (12.2%) (HF-); p < 0.001], angiotensin converting enzyme inhibitors [33 (55.6%) (HF+) vs 725 (41.4%) (HF-); p = 0.025] or nitrates [36 (57.1%) (HF+) vs 495 (28.3%) (HF-); p < 0.001] were more likely to progress to incident HF within one year. Baseline angiotensin II receptor antagonists were only marginally significant [9 (14.3%) (HF+) vs 128 (7.3%) (HF-); p = 0.050). Associations with potassium sparing diuretics/aldosterone antagonists, angiotensin converting enzyme inhibitors, angiotensin II receptor antagonists and nitrates suggest *a priori* coronary artery disease, its risk factors (including hypertension) and its consequence of interest (namely HF), and are consistent with results reported earlier.

As expected, patients developing incident HF within one year of inclusion into either of the three cohorts were more likely to have had their glucose modulating drug introduced against a background of loop diuretic therapy [28 (57.1%) (HF+) vs 694 (20.3%) (HF-), $p < 0.001$ for metformin-sulphonylurea-treated patients; 40 (63.5%) (HF+) vs 485 (27.7%) (HF-), $p < 0.001$ for insulin-treated patients; 15 (53.6%) (HF+) vs 455 (18.0%) (HF-), $p < 0.001$ for thiazolidinedione-treated patients] (data not shown).

Table 3.34 - Comparison of the relative frequency [n (%)] of background and post-treatment drug history among patients treated with metformin-sulphonylurea combination, insulin or thiazolidinedione therapy for a minimum of three months.

	Metformin-sulphonylourea cohort N = 2785	*Insulin cohort N = 1361*	*Thiazolidinedione (TZD) cohort N = 2097*	*p value for the difference across the three cohorts* [a]	*p value for metformin-sulphonylurea cohort vs insulin cohort* [b]	*p value for metformin-sulphonylurea cohort vs TZD cohort* [b]	*p value for insulin cohort vs TZD cohort* [b]
Background p. vasodilators	130 (4.7)	80 (5.9)	67 (3.2)	0.001	0.095	0.010	< 0.001
Post-treatment p. vasodilators	58 (2.1)	54 (4.0)	22 (1.0)	< 0.001	< 0.001	0.005	< 0.001
Background thiazide diuretics	783 (28.1)	283 (20.1)	751 (35.8)	< 0.001	< 0.001	< 0.001	< 0.001
Post-treatment thiazide diuretics	757 (27.2)	412 (30.3)	631 (30.1)	0.035	0.038	0.026	0.910
Background K diuretics / aldosterone antag.	45 (1.6)	28 (2.1)	26 (1.2)	0.168	0.310	0.277	0.058
Post-treatment K diuretics / aldosterone antag.	112 (4.0)	164 (12.0)	61 (2.9)	< 0.001	< 0.001	0.037	< 0.001

K diuretics /aldosterone antag., potassiunm sparing diuretics /aldosterone antagonists; p. vasod5.ilators, peripheral vasodilators;; [a] two-tailed p value (Chi Square test), [b] two-tailed p value (Chi Square test). Tests of the three a priori hypotheses were conducted using Bonferroni adjusted alpha lelves of 0.0167 per test (0.05/3)

	Metformin-sulphonylourea cohort *N = 2785*	*Insulin cohort* *N = 1361*	*Thiazolidinedione (TZD) cohort* *N = 2097*	*p value for the difference across the three cohorts* [a]	*p value for metformin-sulphonylurea cohort vs insulin cohort* [b]	*p value for metformin-sulphonylurea cohort vs TZD cohort* [b]	*p value for insulin cohort vs TZD cohort* [b]
Background NSAIDs	1839 (66.0)	850 (62.5)	1463 (69.8)	< 0.001	0.023	0.006	< 0.001
Post-treatment NSAIDs	892 (32.0)	640 (47.0)	655 (31.2)	< 0.001	< 0.001	0.555	< 0.001
Background dihydropyridine CCBs	908 (32.6)	423 (31.1)	742 (35.4)	0.021	0.324	0.042	0.009
Post-treatment dihydropyridine CCBs	1047 (37.6)	628 (46.1)	732 (34.9)	< 0.001	< 0.001	0.053	< 0.001
Background verapamil	30 (1.1)	18 (1.3)	26 (1.2)	0.760	0.488	0.597	0.832
Post-treatment verapamil	19 (0.7)	18 (1.3)	13 (0.6)	0.049	0.040	0.789	0.032
Background diltiazem	189 (6.8)	140 (10.3)	137 (6.5)	< 0.001	< 0.001	0.726	< 0.001

dihydropyridine CCBs, dihydropyridine calcium channel blockers; NSAIDs, non-steroidal anti-inflamnatory drugs; [a] two-tailed p value (Chi Square test), [b] two-tailed p value (Chi Square test). Tests of the three a priori hypotheses were conducted using Bonferroni adjusted alpha levels of 0.0167 per test (0.05/3)

	Metformin-sulphonylourea cohort *N = 2785*	*Insulin cohort* *N = 1361*	*Thiazolidinedione (TZD) cohort* *N = 2097*	*p value for the difference across the three cohorts* [a]	*p value for metformin-sulphonylurea cohort vs insulin cohort* [b]	*p value for metformin-sulphonylurea cohort vs TZD cohort* [b]	*p value for insulin cohort vs TZD cohort* [b]
Post-treatment diltiazem	175 (6.3)	151 (11.1)	89 (4.2)	< 0.001	< 0.001	0.002	< 0.001
Background beta blockers	1046 (37.6)	479 (35.2)	841 (40.1)	0.013	0.138	0.070	0.004
Post-treatment beta blockers	895 (32.1)	591 (43.4)	663 (31.6)	< 0.001 [a]	< 0.001 [b]	0.700 [b]	< 0.001 [b]
Background vasodilator drugs	15 (0.5)	9 (0.7)	10 (0.5)	0.770 [a]	0.625 [b]	0.765 [b]	0.474 [b]
Post-treatment vasodilator drugs	4 (0.1)	13 (1.0)	1 (0.0)	< 0.001 [a]	< 0.001 [b]	0.399 [c]	< 0.001 [b]
Background centrally acting antiht	24 (0.9)	9 (0.7)	26 (1.2)	0.190 [a]	0.495 [b]	0.194 [b]	0.097 [b]
Post-treatment centrally acting antiht	31 (1.1)	21 (1.5)	18 (0.9)	0.174 [a]	0.243 [b]	0.377 [b]	0.063 [b]

centrally acting antiht, centrally acting antihypertensive drugs; vasodilator drugs, vasodilator antihypertensive drugs; [a] *two-tailed p value (Chi Square test),* [b] *two-tailed p value (Chi Square test). Tests of the three a priori hypotheses were conducted using Bonferroni adjusted alpha levels of 0.0167 per test (0.05/3);* [c] *two-tailed p value (Fisher's exact test). Tests of the three a priori hypotheses were conducted using Bonferroni adjusted alpha levels of 0.0167 per test (0.05/3)*

	Metformin-sulphonylourea cohort *N = 2785*	*Insulin cohort* *N = 1361*	*Thiazolidinedione (TZD) cohort* *N = 2097*	*p value for the difference across the three cohorts* [a]	*p value for metformin-sulphonylurea cohort vs insulin cohort* [b, c]	*p value for metformin-sulphonylurea cohort vs TZD cohort* [b, c]	*p value for insulin cohort vs TZD cohort* [b, c]
Background anbd	4 (0.1)	3 (0.2)	5 (0.2)	0.729 [a]	0.690 [c]	0.511 [c]	1.000 [c]
Post-treatment anbd	2 (0.1)	0 (0)	0 (0)	0.289 [a]	1.000 [c]	0.510 [c]	-
Background aabd	180 (6.5)	97 (7.1)	186 (8.9)	0.006 [a]	0.421 [b]	0.002 [b]	0.068 [b]
Post-treatment aabd	290 (10.4)	247 (18.1)	197 (9.4)	< 0.001 [a]	< 0.001 [b]	0.240 [b]	< 0.001 [b]
Background ACEI	1040 (37.3)	491 (36.1)	1151 (54.9)	< 0.001	0.427	< 0.001	< 0.001
Post-treatment ACEI	1438 (51.6)	916 (67.3)	1179 (56.2)	< 0.001	< 0.001	0.001	< 0.001
Background ARB	226 (8.1)	74 (5.4)	313 (14.9)	< 0.001	0.002	< 0.001	< 0.001

ACEI, angiotensin convering enzyme inhibitors; anbd, adrenergic neurone blocking drugs; aabd, alpha adrenoceptor blocking drugs; ARB, angiotensin II receptor antagonists; [a] *two-tailed p value (Chi Square test),* [b] *two-tailed p value (Chi Square test). Tests of the three a priori hypotheses were conducted using Bonferroni adjusted alpha levels of 0.0167 per test (0.05/3);* [c] *two-tailed p value (Fisher's exact test). Tests of the three a priori hypotheses were conducted using Bonferroni adjusted alpha levels of 0.0167 per test (0.05/3)*

	Metformin-sulphonylourea cohort N = 2785	*Insulin cohort N = 1361*	*Thiazolidinedione (TZD) cohort N = 2097*	*p value for the difference across the three cohorts* [a]	*p value for metformin-sulphonylurea cohort vs insulin cohort* [b]	*p value for metformin-sulphonylurea cohort vs TZD cohort* [b]	*p value for insulin cohort vs TZD cohort* [b]
Post-treatment ARB	391 (14.0)	272 (20.0)	423 (20.2)	< 0.001	< 0.001	< 0.001	0.894
Background renin inhibitors	0 (0)	0 (0)	0 (0)	-	-	-	-
Post-treatment renin inhibitors	0 (0)	0 (0)	0 (0)	-	-	-	-
Background nitrates	584 (21.0)	340 (25.0)	392 (18.7)	< 0.001	0.004	0.049	< 0.001
Post-treatment nitrates	558 (20.0)	455 (33.4)	336 (16.0)	< 0.001	< 0.001	< 0.001	< 0.001
Background other anti-anginal drugs	51 (1.8)	45 (3.3)	46 (2.2)	0.011	0.003	0.369	0.046
Post-treatment other anti-anginal drugs	98 (3.5)	126 (9.3)	53 (2.5)	< 0.001	< 0.001	0.048	< 0.001
Background nitrates	584 (21.0)	340 (25.0)	392 (18.7)	< 0.001	0.004	0.049	< 0.001

ARB, angiotensin II receptor antagonists; [a] *two-tailed p value (Chi Square test),* [b] *two-tailed p value (Ch Square test). Tests of the three a priori hypotheses were conducted using Bonferroni adjusted alpha levels of 0.0167 per test (0.05/3)*

	Metformin-sulphonylourea cohort *N = 2785*	*Insulin cohort* *N = 1361*	*Thiazolidinedione (TZD) cohort* *N = 2097*	*p value for the difference across the three cohorts* [a]	*p value for metformin-sulphonylurea cohort vs insulin cohort* [b]	*p value for metformin-sulphonylurea cohort vs TZD cohort* [b]	*p value for insulin cohort vs TZD cohort* [b]
Post-treatment nitrates	558 (20.0)	455 (33.4)	336 (16.0)	< 0.001	< 0.001	< 0.001	< 0.001
Background other antianginal drugs	51 (1.8)	45 (3.3)	46 (2.2)	0.011	0.003	0.369	0.046
Post-treatment other anti-anginal drugs	98 (3.5)	126 (9.3)	53 (2.5)	< 0.001	< 0.001	0.048	< 0.001

[a] two-tailed p value (Chi Square test), [b] two-tailed p value (Chi Square test). Tests of the three a priori hypotheses were conducted using Bonferroni adjusted alpha levels of 0.0167 per test (0.05/3)

Table 3.35 - Comparison of the relative frequency (n [%]) oprescription of background drug therapy between individuals requiring treatment with loop diuretics and those remaining loop diuretic-free within one year after exposure to metformin-sulphonylurea combination, insulin or thiazolidinedione therapy for a minimum of three months, and having no background loop diuretic therapy at inclusion into their respective cohort.

	Metformin-sulphonylurea cohort			***Insulin cohort***			***Thiazolidinedione cohort***		
	Loop diuretic -treated	***Loop diuretic -free***	***p [a, b]***	***Loop diuretic -treated***	***Loop diuretic -free***	***p [a, b]***	***Loop diuretic -treated***	***Loop diuretic -free***	***p [a, b]***
	N = 131	***N = 2654***		***N = 170***	***N = 1191***		***N = 90***	***N = 2007***	
Background p. vasodilators	12 (9.2)	118 (4.4)	0.013 [a]	15 (8.8)	65 (5.5)	0.081 [a]	3 (3.3)	64 (3.2)	0.763 [b]
Post-treatment p. vasodilators	6 (4.6)	52 (2.0)	0.053 [b]	7 (4.1)	47 (3.9)	0.915 [a]	2 (2.2)	20 (1.0)	0.243 [b]
Background thiazide diuretics	36 (27.5)	747 (28.1)	0.869 [a]	46 (27.1)	237 (19.9)	0.031 [a]	41 (45.6)	710 (35.4)	0.049 [a]
Post-treatment thiazide diuretics	33 (25.2)	724 (27.3)	0.600 [a]	47 (27.6)	365 (30.6)	0.426 [a]	32 (35.6)	599 (29.8)	0.248 [a]
Background K diuretics / aldosterone antag.	2 (1.5)	43 (1.6)	1.000 [b]	6 (3.5)	22 (1.8)	0.149 [b]	3 (3.3)	23 (1.1)	0.098 [b]

K diuretics/aldosterone antag., potassium sparing diuretics/aldosterone antagonists ; p. vasodilators, peripheral vasodilators ; [a] two-sided p value for the statistical difference between loop diuretic-treated and loop diuretic- free patients (Chi Square test); [b]two-sided p value for the statistical difference between loop diuretic-treated and loop diuretic- free patients (Fisher's exact test)

	Metformin-sulphonylurea cohort			*Insulin cohort*			*Thiazolidinedione cohort*		
	Loop diuretic -treated	***Loop diuretic -free***	***p [a, b]***	***Loop diuretic -treated***	***Loop diuretic -free***	***p [a, b]***	***Loop diuretic -treated***	***Loop diuretic -free***	***p [a, b]***
	N = 131	***N = 2654***		***N = 170***	***N = 1191***		***N = 90***	***N = 2007***	
Post-treatment K diuretics / aldosterone antag.	17 (13.0)	95 (3.6)	< 0.001 [a]	56 (32.9)	108 (9.1)	< 0.001 [a]	13 (4.4)	48 (2.4)	< 0.001 [b]
Background NSAIDs	91 (69.5)	1748 (65.9)	0.395 [a]	111 (65.3)	739 (62.0)	0.414 [a]	64 (71.1)	1399 (69.7)	0.776 [a]
Post-treatment NSAIDs	43 (32.8)	849 (32.0)	0.842 [a]	79 (46.5)	561 (47.1)	0.877 [a]	32 (35.6)	623 (31.0)	0.366 [a]
Background dihydropyridine CCBs	55 (42.0)	853 (32.1)	0.019 [a]	63 (37.1)	360 (30.2)	0.072 [a]	40 (44.4)	702 (35.0)	0.066 [a]
Post-treatment dihydropyridine CCBs	56 (42.7)	991 (37.3)	0.212 [a]	75 (44.1)	553 (46.4)	0.571 [a]	36 (40.0)	696 (34.7)	0.300 [a]
Background verapamil	2 (1.5)	28 (1.1)	0.650 [b]	3 (1.8)	15 (1.3)	0.590 [a]	2 (2.2)	24 (1.2)	0.308 [b]

dihydropyridine CCBs, dihydopyridine calcium channel blockers; K diuretics / aldosterone antag., potassium sparing diuretics / aldosterone antagonists; NSAIDs, non-steroidal anti-inflammatory drugs; [a] two-sided p value for the statistical difference between loop diuretic- treated and loop diuretic-free patients (Chi Square test); [b] two-sided p value for the statistical difference between loop diuretic-treated and loop diuretic-free patients (Fisher's exact test)

	Metformin-sulphonylurea cohort			*Insulin cohort*			*Thiazolidinedione cohort*		
	Loop diuretic -treated	***Loop diuretic -free***	***p [a, b]***	***Loop diuretic-treated***	***Loop diuretic -free***	***p [a, b]***	***Loop diuretic -treated***	***Loop diuretic -free***	***p [a, b]***
	N = 131	***N = 2654***		***N = 170***	***N = 1191***		***N = 90***	***N = 2007***	
Post-treatment verapamil	1 (0.8)	18 (0.7)	0.601 [b]	5 (2.9)	13 (1.1)	0.063 [b]	2 (2.2)	11 (0.5)	0.105 [b]
Background diltiazem	19 (14.5)	170 (6.4)	< 0.001 [a]	25 (14.7)	115 (9.7)	0.043 [a]	8 (9.9)	129 (6.4)	0.355 [a]
Post-treatment diltiazem	22 (16.8)	153 (5.8)	< 0.001 [a]	28 (16.5)	123 (10.3)	0.017 [a]	6 (6.7)	83 (4.1)	0.275 [b]
Background beta blockers	64 (48.9)	982 (37.0)	0.006 [a]	71 (41.8)	408 (34.3)	0.055 [a]	34 (37.8)	807 (40.2)	0.645 [a]
Post-treatment beta blockers	49 (37.4)	846 (31.9)	0.186 [a]	94 (55.3)	497 (41.7)	0.001 [a]	37 (41.1)	626 (31.2)	0.048 [a]
Background vasodilator drugs	1 (0.8)	14 (0.5)	0.515 [b]	0 (0)	9 (0.8)	0.612 [b]	1 (1.1)	9 (0.4)	0.356 [b]
Post-treatment vasodilator drugs	0 (0)	4 (0.2)	1.000 [b]	3 (1.8)	10 (0.8)	0.215 [b]	0 (0)	1 (<0.1)	1.000 [b]

vasodilator drugs, vasodilator antithypertensive drugs; [a] two-sided p value for the statistical difference between loop diuretic- treated and loop diuretic- free patients (Chi Square test); [b] two-sided p value for the statistical difference between loop diuretic-treated and loop diuretic-free patients (Fisher's exact test)

	Metformin-sulphonylurea cohort			*Insulin cohort*			*Thiazolidinedione cohort*		
	Loop diuretic -treated	***Loop diuretic -free***	***p*** [a, b]	***Loop diuretic -treated***	***Loop diuretic -free***	***p*** [a, b]	***Loop diuretic -treated***	***Loop diuretic -free***	***p*** [a, b]
	N = 131	***N = 2654***		***N = 170***	***N = 1191***		***N = 90***	***N = 2007***	
Background centrally acting antiht	3 (2.3)	21 (0.8)	0.100 [b]	1 (0.6)	8 (0.7)	1.000 [b]	2 (2.2)	24 (1.2)	0.308 [b]
Post-treatment centrally acting antiht	3 (2.3)	28 (1.1)	0.177 [b]	4 (2.4)	17 (1.4)	0.321 [b]	2 (2.2)	16 (0.8)	0.179 [b]
Background anbd	0 (0)	4 (0.2)	1.000 [b]	1 (0.6)	2 (0.2)	0.330 [b]	1 (1.1)	4 (0.2)	0.197 [b]
Post-treatment anbd	0 (0)	2 (0.1)	1.000 [b]	0 (0)	0 (0)	-	0 (0)	0 (0)	-
Background aabd	11 (8.4)	169 (6.4)	0.356 [a]	22 (12.9)	75 (6.3)	0.002 [a]	10 (11.1)	176 (8.8)	0.445 [a]
Post-treatment aabd	20 (15.3)	270 (10.2)	0.062 [a]	38 (22.4)	209 (17.5)	0.128 [a]	13 (14.4)	184 (9.2)	0.093 [a]

aabd, alpha adrenoceptor blocking drugs; anbd, adrenergic neurone blocking drugs; centrally acting antiht, centrally acting antihypertensive drugs; [a] two-sided p value for the statistical difference between loop diuretic- treated and loop diuretic- free patients (Chi Square test); [b] two-sided p value for the statistical difference between loop diuretic-treated and loop diuretic-free patients (Fisher's exact test)

	Metformin-sulphonylurea cohort			***Insulin cohort***			***Thiazolidinedione cohort***		
	Loop diuretic -treated	***Loop diuretic -free***	***p [a, b]***	***Loop diuretic -treated***	***Loop diuretic -free***	***p [a, b]***	***Loop diuretic -treated***	***Loop diuretic -free***	***p [a, b]***
	N = 131	***N = 2654***		***N = 170***	***N = 1191***		***N = 90***	***N = 2007***	
Background ACEI	50 (38.2)	990 (37.3)	0.841 [a]	71 (41.8)	420 (35.3)	0.099 [a]	53 (58.9)	1098 (54.7)	0.436 [a]
Post-treatment ACEI	75 (57.3)	1363 (51.4)	0.187 [a]	119 (70.0)	797 (66.9)	0.423 [a]	55 (61.1)	1124 (56.0)	0.339 [a]
Background ARB	10 (7.6)	216 (8.1)	0.836 [a]	16 (9.4)	58 (4.9)	0.015 [a]	18 (20.0)	295 (14.7)	0.167 [a]
Post-treatment ARB	24 (18.3)	367 (13.8)	0.148	39 (22.9)	233 (19.6)	0.303	25 (27.8)	398 (19.8)	0.066
Background renin inhibitors	0 (0)	0 (0)	-	0 (0)	0 (0)	-	0 (0)	0 (0)	-
Post-treatment renin inhibitors	0 (0)	0 (0)	-	0 (0)	0 (0)	-	0 (0)	0 (0)	-
Background nitrates	52 (39.7)	532 (20.0)	< 0.001 [a]	60 (35.3)	280 (23.5)	0.001 [a]	25 (27.8)	367 (18.3)	0.024 [a]

ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin II receptor antagonists; [a] two-sided p value for the statistical difference between loop diuretic-treated and loop diuretic- free patients (Chi Square test); [b] two-sided p value for the statistical difference between loop diuretic-treated and loop diuretic-free patients (Fisher's exact test

	Metformin-sulphonylurea cohort			*Insulin cohort*			*Thiazolidinedione cohort*		
	Loop diuretic -treated	***Loop diuretic -free***	***p [a, b]***	***Loop diuretic -treated***	***Loop diuretic -free***	***p [a, b]***	***Loop diuretic -treated***	***Loop diuretic -free***	***p [a, b]***
	N = 131	***N = 2654***		***N = 170***	***N = 1191***		***N = 90***	***N = 2007***	
Post-treatment nitrates	51 (38.9)	507 (19.1)	< 0.001 [a]	86 (50.6)	369 (31.0)	< 0.001 [a]	25 (27.8)	311 (15.5)	0.002 [a]
Background other anti-anginal drugs	0 (0)	51 (1.9)	0.173 [b]	7 (4.1)	38 (3.2)	0.527 [a]	2 (2.2)	44 (2.2)	1.000 [b]
Post-treatment other anti-anginal drugs	6 (4.6)	92 (3.5)	0.463	28 (16.5)	98 (8.2)	0.001	3 (3.3)	50 (2.5)	0.495

[a] two-sided p value for the statistical difference between loop diuretic-treated and loop diuretic-free patients (Chi Square test); [b] two-sided p value for the statistical difference between loop diuretic-treated and loop diuretic- free patients (Fisher's exact test)

3.11.4 Clinical measurements

Table 3.36 summarises clinical measurements for patients belonging to each of the three treatment cohorts, together with two-sided p values for trend across the cohorts, and *post-hoc* pairwise comparisons between the treatment groups. Table 3.37 outlines mean (SD) values, and two-sided p values for the comparison between loop diuretic-treated and -free patients belonging to each of the three treatment cohorts.

Comparing with metformin-sulphonylurea-treated patients, patients prescribed an index thiazolidinedione were characterised by lower baseline mean arterial pressure [99.32 (9.77) vs 100.29 (9.48) mmHg; p = 0.001], lower baseline systolic blood pressure [139.65 (12.92) vs 141.23 (15.70) mmHg; p = 0.001], lower baseline DBP [79.17 (7.92) vs 79.81 (8.64) mmHg; p = 0.032], higher baseline weight [88.97 (17.54) vs 84.67 (16.84) kg; p < 0.001], and higher baseline BMI [31.29 (5.37) vs 30.19 (5.32) kg/m^2 ; p < 0.001] (table 3.36). Higher baseline values for weight and BMI among patients treated with a second or third line thiazolidinedione may reflect the 'end-effect' of several months/years of antecedent (first or second line) sulphonylurea therapy, with their characteristic insulinotropic, weight promoting, effect.

Likewise, comparing thiazolidinedione with insulin-treated patients, the former were characterised by significantly lower baseline systolic blood pressure [139.65 (12.92) vs 141.26 (16.10) mmHg; p = 0.011], higher baseline weight [88.97 (17.54) vs 79.06 (16.51) kg; p < 0.001] and higher baseline BMI [31.29 (5.37) vs 28.43 (5.45) kg/m^2; p < 0.001] (table 3.36).

Index loop diuretic prescribed thiazolidinedione-treated patients were characterised by a significantly higher mean baseline BMI [33.11 (6.54) (LD+) vs 31.21 (5.31) (LD-) kg/m^2; p = 0.002] compared with their index loop diuretic-free counterparts, despite no differences in baseline body weight (table 3.37). BMI is now established as a more precise marker of obesity than body weight. There were no differences in baseline mean arterial pressure and DBP between the two index loop diuretic categories. Mean baseline systolic blood pressure tended to be higher in index loop diuretic-treated patients – with the difference reaching borderline statistical significance [142.35 (13.91) (LD+) vs 139.53 (12.8) (LD); p = 0.048].

Similar observations were reported for baseline mean arterial pressure, DBP and weight among patients on metformin-suphonylurea combination therapy and insulin (without TZD) respectively (table 3.37). Differences in baseline BMI reached statistical significance in either cohort. Baseline systolic blood pressure was significantly higher in loop diuretic prescibed insulin-treated patients; differences in systolic blood pressure did not reach statistical significance in patients on metformin-sulphonylurea combination therapy. Thus index loop diuretic requiring metformin-sulphonylurea-treated patients were characterised by a higher baseline BMI [31.50 (6.07) vs 30.13 (5.28) kg/m^2; p = 0.012]. Likewise, insulin-treated patients characterised by a higher baseline systolic blood pressure [145.07 (16.17) vs 140.73 (16.03) mmHg; p = 0.003] and higher baseline BMI [29.46 (5.60) vs 28.29 (5.42) kg/m^2; p = 0.014] were more prone to ‘oedema’ after index insulin prescription.

Analyzing separately for incident HF events occurring within one year of inclusion into each of the three treatment cohorts yielded no statistical difference in mean baseline systolic blood pressure or baseline body mass index between incident HF subgroups, albeit significantly lower baseline DBP readings for HF prone insulin-treated patients [75.22 (8.70) HF + vs 79.01 (8.78) HF – mmHg; p = 0.001] (data not reproduced in table format).

Table 3.36 - Comparison of mean (SD) values for background and post-treatment clinical measurements among patients treated with metformin-sulphonylurea combination, insulin or thiazolidinedione therapy for a minimum of three months, and no background loop diuretic therapy.

	Metformin-sulphonylourea cohort *N = 2785 (1634 males, 1151 females)*	*Insulin cohort* *N = 1361 (744 males, 617 females)*	*Thiazolidinedione (TZD) cohort* *N = 2097 (1264 males, 833 females)*	*p value for the difference across the three cohorts* [a]	*p value for metformin-sulphonylurea cohort vs insulin cohort* [b]	*p value for metformin-sulphonylurea cohort vs TZD cohort* [b]	*p value for insulin cohort vs TZD cohort* [b]
Baseline MAP (mmHg)	100.29 (9.48)	99.83 (9.55)	99.32 (9.77)	0.003	0.381[c]	0.001[c]	0.303[c]
Post-treatment MAP (mmHg)	99.37 (9.36)	98.61 (9.89)	97.08 (8.22)	< 0.001	0.082[c]	< 0.001[c]	< 0.001[c]
Baseline SBP (mmHg)	141.23 (15.70)	141.26 (16.10)	139.65 (12.92)	0.001	0.999[c]	0.001[c]	0.011[c]
Post treatment SBP (mmHg)	140.51 (15.38)	140.68 (16.21)	137.82 (13.34)	< 0.001	0.952[c]	< 0.001[c]	< 0.001[c]
Baseline DBP (mmHg)	79.81 (8.64)	79.11 (8.53)	79.17 (7.92)	0.016	0.062[c]	0.032[c]	0.979[c]

MAP, mean arterial pressure; SBP, mean systolic blood pressure; [a] *two-tailed p value [One-way analysis of variance (ANOVA)];* [b] *two-tailed p value (pair-wise post-hoc analysis). Tests of these three a priori hypotheses were conducted using the Games-Howell test* [c]

	Metformin-sulphonylourea cohort ***N = 2785 (1634 males, 1151 females)***	***Insulin cohort*** ***N = 1361 (744 males, 617 females)***	***Thiazolidinedione (TZD) cohort*** ***N = 2097 (1264 males, 833 females)***	***p value for the difference across the three cohorts*** [a]	***p value for metformin-sulphonylurea cohort vs insulin cohort*** [b]	***p value for metformin-sulphonylurea cohort vs TZD cohort*** [b]	***p value for insulin cohort vs TZD cohort*** [b]
Post treatment DBP (mmHg)	78.80 (8.67)	77.57 (8.89)	76.71 (8.02)	< 0.001	< 0.001[c]	< 0.001[c]	0.020[c]
Baseline weight (kg)	84.67 (16.84)	79.06 (16.51)	88.97 (17.54)	< 0.001[e]	< 0.001[c, e]	< 0.001[c, e]	< 0.001[c, e]
Post treatment weight (kg)	84.42 (17.15)	81.40 (16.38)	90.04 (17.57)	< 0.001	< 0.001[c]	< 0.001[c]	< 0.001[c]
Baseline BMI (kg/m2)	30.19 (5.32)	28.43 (5.45)	31.29 (5.37)	< 0.001	< 0.001[d]	< 0.001[d]	< 0.001[d]
Post treatment BMI (kg/m^2)	30.15 (5.42)	29.27 (5.43)	31.73 (5.42)	< 0.001	< 0.001[d]	< 0.001[d]	< 0.001[d]

BMI, body mass index; DBP, diastolic blood pressure; [a] two-tailed p value [One-way analysis of variance (ANOVA)]; [b] two-tailed p value (pair-wise post-hoc analysis). Tests of these three a priori hypotheses were conducted using the Games-Howell test [c] and the Tukey-HSD test [d]; [e] differences calculated on log_e transformed data

Table 3.37 - Comparison of mean (SD) values for clinical measurements between individuals requiring treatment with loop diuretics and those remaining loop diuretic-free within one year after exposure to metformin-sulphonylurea combination, insulin or thiazolidinedione therapy for a minimum of three months.

	Metformin-Sulphonylurea cohort			***Insulin cohort***			***Thiazolidinedione cohort***		
	Loop diuretic-treated	***Loop diuretic-free***	***p[a,b]***	***Loop diuretic-treated***	***Loop diuretic-free***	***p[a, b]***	***Loop diuretic-treated***	***Loop diuretic-free***	***p[a, b]***
	N = 131	***N = 2654***		***N = 170***	***N = 1191***		***N = 90***	***N = 2007***	
Baseline MAP (mmHg)	100.98 (9.66)	100.26 (9.48)	0.468[a]	100.71 (9.88)	99.71 (9.50)	0.249[a]	99.53 (8.38)	99.33 (8.00)	0.819[a]
Post-treatment MAP (mmHg)	100.30 (10.04)	99.32 (9.33)	0.304[a]	98.20 (12.07)	98.67 (9.54)	0.611[b]	96.22 (8.07)	97.12 (8.23)	0.334[a]
Baseline SBP (mmHg)	144.33 (15.96)	141.00 (15.68)	0.051[a]	145.07 (16.17)	140.73 (16.03)	0.003[a]	142.35 (13.91)	139.53 (12.86)	0.048[a]
Post treatment SBP (mmHg)	144.30 (17.04)	140.33 (15.28)	0.011[a]	141.73 (18.90)	140.53 (15.79)	0.278[b]	138.29 (14.22)	137.80 (13.30)	0.745[a]
Baseline DBP (mmHg)	79.31 (8.81)	79.83 (8.63)	0.563[a]	78.52 (8.80)	79.20 (8.49)	0.381[a]	78.12 (7.86)	79.22 (7.92)	0.205[a]
Post treatment DBP (mmHg)	78.30 (8.77)	78.82 (8.67)	0.552[a]	76.43 (10.95)	77.74 (8.55)	0.075[b]	75.18 (7.66)	76.78 (8.03)	0.079[a]

DBP, mean diastolic blood pressure; MAP, mean arterial pressure; SBP, mean systolic blood pressure; [a] two-sided p value for the difference between loop diuretic- treated and loop diuretic- free patients (one-way ANOVA); [b]two-sided p value for the difference between loop diuretic-treated and loop diuretic- free patients (Mann-Whtney U test)

	Metformin-Sulphonylurea cohort			***Insulin cohort***			***Thiazolidinedione cohort***		
	Loop diuretic-treated	***Loop diuretic-free***	***p[a]***	***Loop diuretic-treated***	***Loop diuretic-free***	***p[a]***	***Loop diuretic-treated***	***Loop diuretic-free***	***p[a]***
	N = 131	***N = 2654***		***N = 170***	***N = 1191***		***N = 90***	***N = 2007***	
Baseline weight (kg)	85.37 (17.49)	84.64 (16.81)	0.669	81.15 (16.21)	78.77 (16.54)	0.100	91.01 (19.81)	88.88 (17.44)	0.278
Post treatment weight (kg)	88.63 (18.76)	84.22 (17.06)	0.013	83.46 (17.31)	81.11 (16.23)	0.098	93.55 (19.44)	89.88 (17.47)	0.073
Baseline BMI (kg/m^2)	31.50 (6.07)	30.13 (5.28)	0.012	29.46 (5.60)	28.29 (5.42)	0.014	33.11 (6.54)	31.21 (5.31)	0.002
Post treatment BMI (kg/m^2)	32.39 (6.06)	30.05 (5.37)	<0.001	30.56 (6.25)	29.09 (5.30)	0.002	34.27 (6.61)	31.61 (5.33)	<0.001

BMI, mean body mass index; ; [a] two-sided p value for the difference between loop diuretic-treated and loop diuretic-free patients (one-way ANOVA)

3.11.5 Haematology and biochemistry

Inspection of baseline haematocrit, biochemistry profile and thyrotropin concentrations across the metformin-sulphonylurea, insulin and thiazolidinedione cohorts yielded unexpected findings. Patients requiring an index thiazolidinedione prescription were characterised by lower baseline total cholesterol [4.46 (0.93) vs 4.84 (1.18) mmol/L; $p < 0.001$], lower baseline low density lipoprotein cholesterol (LDL-C) [2.29 (0.90) vs 2.50 (1.04) mmol/L; $p < 0.001$], higher baseline serum sodium [136.68 (2.73) vs 138.39 (2.86) mmol/L; $p = 0.001$], higher baseline estimated glomerular filtration rate [96.40 (35.91) vs 91.54 (36.13) mls/min/1.73 m^2; $p < 0.001$], and a higher baseline serum albumin [44.00 (2.88) vs 43.51 (3.55) g/L; $p < 0.001$] (table 3.38) compared with their metformin-sulphonylurea-treated counterparts. Higher baseline values for estimated glomerular filtration rate and serum albumin for thiazolidinedione-treated patients are perhaps rather surprising, but could stem from an *a priori* tendency to avoid metformin and/or sulphonylureas in patients with impaired renal and/or liver function, shifting mean (SD) values for these variables. Lower baseline serum total cholesterol concentrations at index thiazolidinedione prescription could perhaps reflect a metformin-associated benefical effect on lipid status, as reported in a meta-analysis by Wulffele et al [607].

Comparing thiazolidinedione and insulin-treated patients, the former were characterised by a higher baseline haematocrit [42.26 (3.68) vs 40.22 (4.59) %; $p < 0.001$], a lower baseline HbA1c [8.89 (1.37) vs 9.67 (1.82) %; $p < 0.001$], lower baseline total cholesterol [4.46 (0.93) vs 4.94 (1.21) mmol/L; $p < 0.001$], lower baseline LDL-C [2.29 (0.90) vs 2.60 (1.01) mmol/L; $p < 0.001$], higher baseline

alanine aminotransferase (ALT) [33.51 (19.56) vs 31.84 (24.66); $p < 0.001$], higher baseline serum sodium [138.68 (2.73) vs 137.32 (3.18); $p < 0.001$], higher baseline estimated glomerular filtration rate (eGFR) [96.40 (35.91) vs 79.39 (31.11) mls/min/1.73 m^2; $p < 0.001$], higher baseline TSH [2.03 (1.33) vs 1.99 (1.45) mIU/L; $p = 0.017$], higher baseline serum albumin [44.00 (2.88) vs 41.53 (4.81) g/dL; $p < 0.001$] and a lower baseline serum creatinine [88.16 (20.70) vs 94.83 (33.57); $p < 0.001$] (table 3.38). Lower baseline values for serum haematocrit for insulin-treated patients could stem from a tendency to switch patients from thiazolidinediones to insulin therapy in the face of fluid overload. It is perhaps not entirely surprising that patients prescribed insulin therapy are prone to poorer renal function at baseline – probably reflecting the gradual deterioration characteristic of patients with poorly controlled T2DM (higher HbA1c, total cholesterol and LDL-C concentrations). Given the reported association between ALT and visceral fat accumulation, higher baseline ALT for thiazolidinedione-treated patients could stem from a tendency to prescribe these 'third line' insulin sensitizers in patients with surrogate markers of insulin resistance. As a corollary, a lower mean baseline ALT in insulin-treated T2DM patients could reflect the 'end result' of thiazolidinedione prescription in patients moving on to 'fourth line' insulin therapy.

As outlined in table 3.39, patients requiring an index loop diuretic within one year of index thiazolidinedione therapy were characterised by significantly lower baseline values for serum albumin [42.54 (3.69) (LD+) vs 44.06 (2.82) (LD-) g/dL; $p < 0.001$] and estimated glomerular filtration rates [67.65 (21.21) (LD+) vs 76.61 (19.03) (LD-) mls/min/1.73m^2; $p < 0.001$] despite no differences in baseline serum creatinine [93.80 (28.18) (LD+) vs 87.93 (20.30) (LD-) μmol/L; $p = 0.152$]. This is

consistent with the observation of lower haematocrit values (a surrogate measure of haemodilution, and hence fluid balance) for such patients [40.93 (4.26) (LD+) vs 42.32 (3.64) (LD-) %; p = 0.001]. Index loop diuretic-treated TZD patients had lower baseline ALT values than their loop diuretic-free counterparts [28.60 (16.07) (LD+) vs 33.72 (19.67) IU/L (LD-); p = 0.003]. Given the reported association between prevalent ALT and visceral fat accumulation, this observation surprisingly seems to suggest that insulin sensitivity is a predisposing factor to thiazolidinedione-associated fliud retention. There were no differences in baseline HbA1c, sodium, total cholesterol (and its lipoprotein fractions), triglycerides and TSH (albeit a trend towards higher TSH values for loop diuretic-treated patients [2.37 (1.52) (LD+] vs 2.01 (1.32) (LD-) mIU/L; p = 0.054] (table 3.39). Indeed, subclinical hypothyroidism has been associated with increased capillary permeability to protein in a small clinical study of nine female patients [608]. Whether this association holds true for TSH values within the reference range remains to be determined.

Analyzing for patients both control cohorts, loop diuretic-treated patients were likewise characterised by a lower baseline haematocrit, estimated glomerular filtration rate and serum albumin and a significantly higher serum creatinine than their loop diuretic-free counterparts (table 3.39). There were no differences in baseline lipid profile and thyrotropin concentrations for either cohort. Insulin-treated patients requiring an index loop diuretic were characterised by better glycaemic control (lower HbA1c). Individuals treated with an index loop diuretic after index metformin-sulphonylourea combination therapy were uniquely characterised by a lower baseline serum sodium concentration, suggesting a role for altered sodium haemodynamics in such patients at a relatively early stage of T2DM. An alteration in

the prevalent sodium milieu in ‘oedema prone’ patients could be masked by other (stronger) contributory factors in thiazolidinedione and insulin-treated patients whose T2DM is more likely to be complicated by macrovascular and microvascular disease

Table 3.38 - Comparison of mean (SD) values for heamatology and biochemistry results of patients treated with metformin-sulphonylurea combination, insulin and thiazolidinedione therapy for at least three months, and having no background loop diuretic therapy at inclusion into their respective cohort.

	Metformin-sulphonylourea cohort N = 2785	*Insulin cohort N = 1361*	*Thiazolidinedione (TZD) cohort N = 2097*	*p value for the difference across the three cohorts*[a]	*p value for metformin-sulphonylurea cohort vs insulin cohort*[b]	*p value for metformin-sulphonylurea cohort vs TZD cohort*[b]	*p value for insulin cohort vs TZD cohort*[b]
Baseline haematocrit (%)	42.10 (3.95)	40.22 (4.59)	42.26 (3.68)	< 0.001	< 0.001[c]	0.398 [c]	< 0.001 [c]
Post-treatment haematocrit (%)	40.93 (4.40)	40.46 (4.67)	40.56 (4.32)	0.006	0.011[d]	0.040 [d]	0.812 [d]
Baseline HbA1c (%)	8.91 (1.54)	9.67 (1.82)	8.89 (1.37)	< 0.001[e]	< 0.001[c, e]	0.928[c, e]	< 0.001[c, e]
Post treatment HbA1c (%)	7.83 (1.47)	8.57 (1.55)	8.23 (1.47)	< 0.001[e]	< 0.001[c, e]	< 0.001[c, e]	< 0.001[c, e]
Baseline total cholesterol (mmol/L)	4.84 (1.18)	4.94 (1.21)	4.46 (0.93)	< 0.001[e]	0.057[c, e]	< 0.001[c, e]	< 0.001[c, e]
Post treatment total cholesterol (mmol/L)	4.70 (1.13)	4.87 (1.26)	4.61 (1.05)	< 0.001[e]	0.001[c, e]	0.108[c, e]	< 0.001[c, e]

HbA1c, glycosylated haemoglobin; [a] two-tailed p value [one-way analysis of variance (ANOVA)]; [b] two-tailed p value (pair-wise post-hoc analysis). Tests of these three a priori hypotheses were conducted using the Games-Howell test [c] and the Tukey-HSD test [d]; [e] differences calculated on log_e transformed data

	Metformin-sulphonylourea cohort *N = 2785*	*Insulin cohort* *N = 1361*	*Thiazolidinedione (TZD) cohort* *N = 2097*	*p value for the difference across the three cohorts* [a]	*p value for metformin-sulphonylurea cohort vs insulin cohort* [b]	*p value for metformin-sulphonylurea cohort vs TZD cohort* [b]	*p value for insulin cohort vs TZD cohort* [b]
Baseline HDL-C (mmol/L)	1.20 (0.33)	1.21 (0.36)	1.21 (0.31)	0.074 [e]	0.856 [c, e]	0.055 [c, e]	0.470 [c, e]
Post treatment HDL-C (mmol/L)	1.21 (0.34)	1.30 (0.40)	1.29 (0.32)	< 0.001 [e]	< 0.001 [c, e]	< 0.001 [c, e]	0.485 [c, e]
Baseline LDL-C (mmol/L)	2.50 (1.04)	2.60 (1.01)	2.29 (0.90)	< 0.001 [f]	0.112 [c, f]	< 0.001 [c, f]	< 0.001 [c, f]
Post treatment LDL-C (mmol/L)	2.37 (0.91)	2.44 (1.01)	2.15 (0.80)	< 0.001 [f]	0.142 [c, f]	< 0.001 [c, f]	< 0.001 [c, f]
Baseline triglycerides (mmol/L)	2.71 (1.83)	2.73 (1.83)	2.60 (1.67)	0.358 [e]	0.989 [c, e]	0.351 [c, e]	0.606 [c, e]
Post-treatment triglycerides (mmol/L)	2.37 (1.55)	2.35 (1.63)	2.41 (1.60)	0.223 [e]	0.408 [d, e]	0.853 [d, e]	0.206 [d, e]

HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; [a] two-tailed p value [one-way analysis of variance (ANOVA)]; [b] two-tailed p value (pair-wise post-hoc analysis). Tests of these three a priori hypotheses were conducted using the Games-Howell test [c] and the Tukey-HSD test [d]; [e] differences calculated on log_e transformed data; [f] differences calculated on square root transformed data

	Metformin-sulphonylourea cohort *N = 2785*	*Insulin cohort* *N = 1361*	*Thiazolidinedione (TZD) cohort* *N = 2097*	*p value for the difference across the three cohorts* [a]	*p value for metformin-sulphonylurea cohort vs insulin cohort* [b]	*p value for metformin-sulphonylurea cohort vs TZD cohort* [b]	*p value for insulin cohort vs TZD cohort* [b]
Baseline ALT (IU/L)	33.15 (20.68)	31.84 (24.66)	33.51 (19.56)	< 0.001[e]	0.001 [c, e]	0.180 [c, e]	< 0.001[c, e]
Post treatment ALT (IU/L)	31.19 (21.94)	28.19 (22.08)	28.59 (16.97)	< 0.001[e]	< 0.001 [c, e]	0.019 [c, e]	0.001[c, e]
Baseline sodium (mmol/L)	138.39 (2.86)	137.32 (3.18)	138.68 (2.73)	< 0.001	< 0.001 [c]	0.001 [c]	< 0.001[c]
Post treatment sodium (mmol/L)	138.96 (2.96)	138.45 (3.16)	139.25 (2.68)	< 0.001	< 0.001 [c]	0.002 [c]	< 0.001[c]
Baseline eGFR (mls/min/1.72 m^2)	91.54 (36.13)	79.39 (31.11)	96.40 (35.91)	< 0.001[e]	< 0.001 [c, e]	< 0.001 [c, e]	< 0.001[c, e]
Post treatment eGFR (mls/min/1.72 m^2)	85.80 (34.38)	75.83 (30.82)	95.77 (36.42)	< 0.001[e]	< 0.001 [d, e]	< 0.001 [d, e]	< 0.001[d, e]

ALT, alanine aminotransferase; eGFR, estimated glomerular filtration rate; [a] two-tailed p value [one-way analysis of variance (ANOVA)]; [b] two-tailed p value (pair-wise post-hoc analysis). Tests of these three a priori hypotheses were conducted using the Games-Howell test [c] and the Tukey-HSD test [d]; [e] differences calculated on log_e transformed data

	Metformin-sulphonylourea cohort *N = 2785*	*Insulin cohort* *N = 1361*	*Thiazolidinedione (TZD) cohort* *N = 2097*	*p value for the difference across the three cohorts* [a]	*p value for metformin-sulphonylurea cohort vs insulin cohort* [b]	*p value for metformin-sulphonylurea cohort vs TZD cohort* [b]	*p value for insulin cohort vs TZD cohort* [b]
Baseline TSH (mIU/L)	2.00 (1.52)	1.99 (1.45)	2.03 (1.33)	< 0.00 [e]	0.638 [c,e]	0.023 [c,e]	0.017 [c,e]
Post treatment TSH (mIU/L)	2.18 (1.72)	2.08 (1.73)	2.15 (1.47)	0.015 [e]	0.029 [d,e]	0.428 [d,e]	0.004 [d,e]
Baseline serum albumin (g/L)	43.51 (3.55)	41.53 (4.81)	44.00 (2.88)	< 0.001 [e]	< 0.001 [c, e]	< 0.001 [c, e]	< 0.001 [c, e]
Post treatment serum albumin (g/L)	43.35 (3.52)	41.20 (4.24)	43.93 (2.86)	< 0.001 [e]	< 0.001 [c, e]	< 0.001 [c, e]	< 0.001 [c, e]
Baseline serum creatinine (µmol/L)	87.00 (20.88)	94.83 (33.57)	88.16 (20.70)	< 0.001 [f]	< 0.001 [d, f]	0.139 [d, f]	< 0.001 [d, f]
Post treatment serum creatinine (µmol/L)	90.75 (27.74)	102.58 (41.94)	89.45 (24.69)	< 0.001 [f]	< 0.001 [d, f]	0.157 [d, f]	< 0.001 [d, f]

TSH, thyroid stimulating hormone; [a] two-tailed p value [one-way analysis of variance (ANOVA)]; [b] two-tailed p value (pair-wise post-hoc analysis). Tests of these three a priori hypotheses were conducted using the Games-Howell test [c] and the Tukey-HSD test [d]; [e] differences calculated on square root transformed data; [f] differences calculated on reciprocally transformed data

Table 3.39 - Comparison of mean (SD) values for blood investigations between individuals requiring treatment with loop diuretics and those remaining loop diuretic-free within one year after exposure to metformin-sulphonylurea combination, insulin or thiazolidinedione therapy for a minimum of three months.

	Metformin-Sulphonylurea cohort			***Insulin cohort***			***Thiazolidinedione cohort***		
	Loop diuretic -treated	***Loop diuretic-free***	***p[a, b]***	***Loop diuretic -treated***	***Loop diuretic-free***	***p[a, b]***	***Loop diuretic -treated***	***Loop diuretic-free***	***p[a, b]***
	N = 131	***N = 2654***		***N = 170***	***N = 1191***		***N = 90***	***N = 2007***	
Baseline haematocrit (%)	41.21 (4.31)	42.14 (3.93)	0.037[a]	38.87 (5.21)	40.43 (4.45)	0.001[a]	40.93 (4.26)	42.32 (3.64)	0.001[a]
Post-treatment haematocrit (%)	39.14 (5.03)	41.02 (4.34)	<0.001[a]	38.62 (5.26)	40.74 (4.51)	<0.001[a]	38.78 (4.73)	40.65 (4.28)	<0.001[a]
Baseline HbA1c (%)	8.82 (1.45)	8.91 (1.54)	0.563[a]	9.23 (1.91)	9.71 (1.80)	0.002[a]	8.78 (1.47)	8.90 (1.37)	0.432[a]
Post treatment HbA1c (%)	7.95 (1.44)	7.82 (1.48)	0.333[a,c]	8.49 (1.69)	8.58 (1.53)	0.330[a,c]	8.06 (1.61)	8.24 (1.46)	0.180[a,c]
Baseline total cholesterol (mmol/L)	4.85 (1.19)	4.84 (1.18)	0.936[a]	4.98 (1.36)	4.94 (1.19)	0.676[a,c]	4.38 (0.99)	4.46 (0.93)	0.419[a]
Post treatment total cholesterol (mmol/L)	4.75 (1.23)	4.69 (1.12)	0.526[b]	4.79 (1.30)	4.89 (1.26)	0.314[a,c]	4.39 (1.03)	4.62 (1.05)	0.028[a,c]

HbA1c, glycosylated haemoglobin; [a] two-tailed p value for the difference between loop diuretic-treated and loop diuretic- free patients (one-way ANOVA); [b]two-tailed p value for the difference between loop diuretic-treated and loop diuretic-free patients (Mann Whitney U test); [c] differences calculated on $\log_e$ transformed data

	Metformin-Sulphonylurea cohort			*Insulin cohort*			*Thiazolidinedione cohort*		
	Loop diuretic-treated	***Loop diuretic-free***	***p[a, b]***	***Loop diuretic-treated***	***Loop diuretic-free***	***p[a, b]***	***Loop diuretic-treated***	***Loop diuretic-free***	***p[a, b]***
	N = 131	***N = 2654***		***N = 170***	***N = 1191***		***N = 90***	***N = 2007***	
Baseline HDL-C (mmol/L)	1.20 (0.32)	1.20 (0.33)	0.933[a,c]	1.26 (0.35)	1.20 (0.36)	0.067[a,c]	1.27 (0.32)	1.21 (0.31)	0.126[a,c]
Post treatment HDL-C (mmol/L)	1.23 (0.43)	1.21 (0.34)	0.555[b]	1.24 (0.38)	1.30 (0.40)	0.040[a,c]	1.31 (0.30)	1.29 (0.32)	0.501[a,c]
Baseline LDL-C (mmol/L)	2.37 (1.30)	2.51 (1.03)	0.110[b,c]	2.63 (1.03)	2.59 (1.01)	0.784[a]	2.16 (0.90)	2.29 (0.90)	0.264[a]
Post treatment LDL-C (mmol/L)	2.34 (0.87)	2.37 (0.92)	0.778[a]	2.44 (1.04)	2.44 (1.01)	0.974[a]	2.07 (0.81)	2.15 (0.80)	0.449[a]
Baseline triglycerides (mmol/L)	2.99 (2.39)	2.70 (1.81)	0.833[b,c]	2.76 (1.86)	2.73 (1.83)	0.838[a,c]	2.57 (2.04)	2.60 (1.65)	0.475[a,c]
Post-treatment triglycerides (mmol/L)	2.39 (1.63)	2.37 (1.55)	0.904[a,c]	2.41 (1.54)	2.34 (1.65)	0.276[a,c]	2.24 (1.29)	2.42 (1.61)	0.605[a,c]

HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; [a] two-sided p value for the difference between loop diuretic-treated and loop diuretic-free patients (one-way ANOVA); [b] two-sided p value for the difference between loop diuretic-treated and loop diuretic-free patients (Mann Whitney U test); [c] differences calculated on $\log_e$ transformed data; [f] differences calculated on square root transformed data

	Metformin-Sulphonylurea cohort			*Insulin cohort*			*Thiazolidinedione cohort*		
	Loop diuretic-treated	***Loop diuretic-free***	***p*[a, b]**	***Loop diuretic-treated***	***Loop diuretic-free***	***p*[a, b]**	***Loop diuretic-treated***	***Loop diuretic-free***	***p*[a, b]**
	N = 131	***N = 2654***		***N = 170***	***N = 1191***		***N = 90***	***N = 2007***	
Baseline ALT (IU/L)	31.43 (18.18)	33.23 (20.78)	0.316 [a, c]	31.98 (21.69)	31.82 (25.11)	0.714 [a, c]	28.60 (16.07)	33.72 (19.67)	0.003 [a, c]
Post treatment ALT (IU/L)	28.19 (19.33)	31.34 (22.06)	0.045 [a, c]	26.94 (18.89)	28.38 (22.52)	0.409 [a, c]	27.79 (23.70)	28.63 (16.59)	0.157 [a, c]
Baseline sodium (mmol/L)	137.79 (3.38)	138.42 (2.83)	0.028[a]	137.59 (3.54)	137.28 (3.13)	0.125[b]	138.80 (3.09)	138.68 (2.71)	0.613[a]
Post treatment sodium (mmol/L)	138.44 (3.66)	138.98 (2.92)	0.220[b]	138.35 (3.18)	138.46 (3.15)	0.671[a]	139.61 (2.88)	139.23 (2.67)	0.183[a]
Baseline eGFR (mls/min/1.73 m^2)	69.60 (18.53)	77.01 (19.71)	0.009 [a, c]	63.07 (20.48)	71.34 (19.94)	<0.001 [a, c]	67.65 (21.21)	76.61 (19.03)	<0.001 [a, c]
Post treatment eGFR (mls/min/1.73 m^2)	67.57 (20.74)	75.09 (19.74)	0.232 [a, c]	58.33 (20.22)	68.45 (19.90)	<0.001 [a, c]	66.80 (22.58)	76.21 (20.08)	0.002a, [c]

ALT, alanine aminotransferase; eGFR,, estimated glomerular filtration rate; [a] two-sided p value for the difference between loop diuretic-treated and loop diuretic-free patients (one-way ANOVA); [b] two-sided p value for the difference between loop diuretic-treated and loop diuretic-free patients (Mann Whitney U test); [c] differences calculated on log_e transformed data

	Metformin-Sulphonylurea cohort			*Insulin cohort*			*Thiazolidinedione cohort*		
	Loop diuretic-treated	***Loop diuretic-free***	***p*** [a, b]	***Loop diuretic-treated***	***Loop diuretic-free***	***p*** [a, b]	***Loop diuretic-treated***	***Loop diuretic-free***	***p*** [a, b]
	N = 131	***N = 2654***		***N = 170***	***N = 1191***		***N = 90***	***N = 2007***	
Baseline TSH (mIU/L)	1.92 (1.37)	2.00 (1.53)	0.381 [a, c]	1.84 (1.15)	2.01 (1.50)	0.255 [a, c]	2.37 (1.52)	2.01 (1.32)	0.054 [b, c]
Post treatment TSH (mIU/L)	2.21 (1.35)	2.18 (1.74)	0.699 [a, c]	2.05 (1.79)	2.08 (1.72)	0.652 [a, c]	2.44 (2.02)	2.13 (1.43)	0.526 [b, c]
Baseline serum albumin (g/L)	41.97 (4.12)	43.59 (3.50)	<0.001 [a, c]	39.44 (5.35)	41.85 (4.65)	<0.001 [a, c]	42.54 (3.69)	44.06 (2.82)	<0.001 [a, c]
Post treatment serum albumin (g/L)	41.61 (3.71)	43.44 (3.49)	<0.001 [a, c]	39.79 (4.59)	41.42 (4.14)	<0.001 [a, c]	42.84 (3.14)	43.98 (2.84)	0.001 [a, c]
Baseline serum creatinine (µmol/L)	93.65 (32.84)	86.67 (20.07)	0.006 [a, d]	104.89 (42.82)	93.29 (31.67)	<0.001 [a, d]	93.80 (28.18)	87.93 (20.30)	0.152 [b, d]
Post treatment serum creatinine (µmol/L)	103.24 (55.72)	90.10 (25.32)	<0.001 [a, d]	118.05 (55.86)	100.20 (38.85)	<0.001 [a, d]	99.15 (33.44)	88.98 (24.10)	0.001 [a, d]

TSH, serum Thyroid Stimulating Hormone; [a] two-sided p value for the difference between loop diuretic-treated and loop diuretic- free patients (one-way ANOVA); [b] two-sided p value for the difference between loop diuretic-treated and loop diuretic-free patients (Mann Whitney U test); [c] differences calculated on square root transformed data; [d] differences calculated on reciprocally transformed dat

3.11.6 Echocardiography

Echocardiographic data were available for only a small subset of patients within each of the three treatment cohorts, as outlined in tables 3.40 and 3.41 below. Nonetheless, this study analysed baseline and post-treatment echocardiographic parameters in this subgroup of patients having echocardiographic data before and after prescription of index thiazolidinedione, metformin-sulphonylurea combination and insulin therapy. Baseline interventricular septum wall thickness, left ventricular posterior wall thickness and left ventricular mass for thiazolidinedione-treated patients did not significantly differ from corresponding values for metformin-sulphonylurea or insulin prescribed subjects (table 3.40).

In general, loop diuretic-treated patients were characterised by higher mean (SD) values for each of the baseline echocardiographic parameters. Thus, ‘oedema prone’ thiazolidinedione-treated patients were characterised by a significantly higher baseline left ventricular mass compared with their index loop diuretic-free counterparts [288.52 (81.78) (LD+) vs 234.54 (77.00) (LD-) g; $p = 0.029$] (table 3.41). Likewise, statistical differences were observed between index loop diuretic categories for metformin-sulphonylurea-treated patients [301.35 (50.52) (LD+) vs 235.33 (74.44) (LD-); $p = 0.010$] but not among patients administered insulin. Baseline interventricular septum thickness was significantly higher among patients prescribed an index loop diuretic after index metformin-sulphonylurea combination therapy [1.51 (0.16) (LD+) vs 1.27 (0.28) (LD); $p = 0.005$]. Such differences, although noticeable in the insulin and thiazolidinedione cohorts, did not reach statistical significance, possibly as a result of small sample size (table 3.41).

Table 3.40 - Comparison of mean (SD) values for echocardiographic parameters for a subset of patients [a] treated with metformin-sulphonylurea combination, insulin and thiazolidinedione therapy for at least three months, and having no background loop diuretic therapy at inclusion into their respective cohort.

	Metformin-sulphonylourea cohort	*Insulin cohort*	*Thiazolidinedione (TZD) cohort*	*p value for the difference across the three cohorts [b, c]*	*p value for metformin-sulphonylurea cohort vs insulin cohort [d]*	*p value for metformin-sulphonylurea cohort vs TZD cohort [d]*	*p value for insulin cohort vs TZD cohort [d]*
Baseline IVS thickness (cm)	***n = 162*** [a] 1.29 (0.28)	***n = 104*** [a] 1.25 (0.28)	***n = 129*** [a] 1.31 (0.26)	0.247 [b, f]	0.419 [d, f]	0.310 [d, f]	0.100 [d, f]
Post-treatment IVS thickness (cm)	***n = 173*** [a] 1.31 (0.27)	***n = 201*** [a] 1.32 (0.28)	***n = 111*** [a] 1.36 (0.27)	0.309 [b, f]	0.730 [d, f]	0.142 [d, f]	0.215 [d, f]
Baseline LVPW thickness (cm)	***n = 150*** [a] 1.12 (0.22)	***n = 90*** [a] 1.18 (0.53)	***n = 110*** [a] 1.19 (0.38)	0.159 [b, g]	0.732 [d, g]	0.137 [d, g]	0.064 [d, g]
Post-treatment LVPW thickness (cm)	***n = 143*** [a] 1.17 (0.40)	***n = 178*** [a] 1.16 (0.24)	***n = 90*** [a] 1.28 (0.50)	0.028 [b, g]	0.605 [d, g]	0.010 [d, g]	0.028 [d, g]
Baseline LV mass (g)	***n = 146*** [a] 238.50 (74.70)	***n = 87*** [a] 248.55 (161.07)	***n = 108*** [a] 238.04 (78.07)	0.980 [c]	0.806 [e]	0.927 [e]	0.985 [e]
Post-treatment LV mass (g)	***n = 139*** [a] 250.32 (109.36)	***n = 173*** [a] 246.88 (74.49)	***n = 88*** [a] 275.63 (141.07)	0.225 [b, f]	0.675 [d, f]	0.098 [d, f]	0.160 [d, f]

IVS, interventricular septum; LV, left ventricle; LVPW, left ventricular posterior wall; [a] subset of the whole cohort containing echocardiographic data; [b] two-tailed p value (one-way ANOVA); [c] two-tailed p value [Kruskal-Wallis test]; [d] pair-wise post-hoc parametric tests were conducted using the Tukey-HSD test; [e] Mann-Whitney U test (post-hoc analysis) - two-tailed p values were Bonferroni corrected; [f] differences calculated on $\log_e$ transformed data; [g] differences calculated on reciprocally transformed data

Table 3.41 - Comparison of mean (SD) values for baseline and post-treatment bechocardiographic parameters between individuals requiring treatment with loop diuretics and those remaining loop diuretic-free within one year after exposure to metformin-sulphonylurea combination, insulin or thiazolidinedione therapy for a minimum of three months, and no background loop diuretic therapy

	Metformin-sulphonylurea cohort			***Insulin cohort***			***Thiazolidinedione cohort***		
	Loop diuretic - treated	***Loop diuretic -free***	***p*** [b, c]	***Loop diuretic- treated***	***Loop diuretic -free***	***p*** [b, c]	***Loop diuretic- treated***	***Loop diuretic -free***	***p*** [b, c]
Baseline IVS thickness (cm)	***n = 7*** [a] 1.51 (0.16)	***n =155*** [a] 1.27 (0.28)	0.005 [b, d]	***n = 22*** [a] 1.31 (0.35)	***n = 82*** [a] 1.23 (0.25)	0.356 [b]	***n = 8*** [a] 1.38 (0.28)	***n = 121*** [a] 1.30 (0.26)	0.394 [c]
Post-treatment IVS thickness (cm)	***n = 17*** [a] 1.33 (0.28)	***n = 156*** [a] 1.30 (0.27)	0.712 [c]	***n = 42*** [a] 1.29 (0.26)	***n = 159*** [a] 1.33 (0.28)	0.403 [c]	***n = 9*** [a] 1.39 (0.20)	***n = 102*** [a] 1.35 (0.28)	0.737 [c]
Baseline LVPW thickness (cm)	***n = 7*** [a] 1.20 (0.25)	***n = 143*** [a] 1.11 (0.22)	0.321 [c, d]	***n = 18*** [a] 1.25 (0.44)	***n = 72*** [a] 1.16 (0.55)	0.269 [c, e]	***n = 7*** [a] 1.30 (0.28)	***n = 103*** [a] 1.18 (0.39)	0.201 [c, e]
Post-treatment LVPW thickness (cm)	***n = 16*** [a] 1.37 (0.73)	***n = 127*** [a] 1.15 (0.33)	0.115 [b]	***n = 38*** [a] 1.12 (0.23)	***n = 140*** [a] 1.16 (0.24)	0.362 [c]	***n = 10*** [a] 1.60 (1.17)	***n = 80*** [a] 1.24 (0.33)	0.367 [b]
Baseline LV mass (g)	***n = 7*** [a] 301.35 (50.52)	***n = 139*** [a] 235.33 (74.44)	0.010 [b]	***n = 18*** [a] 263.54 (90.90)	***n = 69*** [a] 244.64 (175.13)	0.212 [c, e]	***n = 7*** [a] 288.52 (81.78)	***n = 101*** [a] 234.54 (77.00)	0.029 [b]
Post-treatment LV mass (g)	***n = 15*** [a] 333.57 (188.56)	***n = 124*** [a] 240.25 (91.78)	0.003 [b]	***n = 38*** [a] 250.83 (59.58)	***n = 135*** [a] 245.77 (78.33)	0.493 [b]	***n = 9*** [a] 390.22 (343.23)	***n = 79*** [a] 62.58 (91.79)	0.213 [b]

IVS, interventricular septum; LVPW, left ventricular posterior wall; [a] subset of the whole cohort containing echocardiographic data; [b] two-tailed p value for the statistical difference between loop diuretic- treated and loop diuretic- free patients (Mann Whitney U test); [c] two-tailed p value for the statistical difference betwen loop diuretic-treated and loop diuretic-free patients (one-way ANOVA); [d] differences calculated on $\log_e$ transformed data; [e] differences calculated on reciprocally transformed data

3.12 Logistic regression model: predicting risk factors for index loop diuretic prescription required within one year after index metformin-sulphonylurea combination or thiazolidinedione therapy

3.12.1 Univariate logistic regression

Given the similar proportions of patients requiring an index loop diuretic prescription after incident metformin-sulphonylurea combination or thiazolidinedione therapy, I opted to investigate whether index thiazolidinedione therapy is associated with an increased risk of fluid retention compared with metformin-sulphonylureas combination therapy on multivariate analysis. Patients on insulin therapy were not included as a comparator cohort in this logistic regression analysis, given that they are likely to represent a more diseased cohort, with potentially different confounding factors influencing index loop diuretic prescription, as suggested by the results of this study's descriptive analysis. None of the patients were being treated with a baseline renin inhibitor at inclusion into the treatment cohort, and thus could not be included in univariate or multivariate analysis. Categorical covariates were dummy coded, using non-exposure to the categorical variable of interest as the reference group (and conversely, exposure as the indicator group). Univariate analysis found that index loop diuretic prescription within one year of inclusion into either the metformin-sulphonylurea/metformin cohort was significantly associated with the following characteristics (tables 3.42 and 3.43):

Demographics

- age in years [OR 1.047 (95% CI 1.033, 1.061); p < 0.001]
- diabetes duration in years *(square root transformed data)* [OR 1.290 (95% CI 1.131, 1.472); p < 0.001]
- female gender [OR 1.392 (95% CI 1.062, 1.824); p = 0.016]

Past medical history

- baseline macrovascular disease [OR 2.459 (95% CI 1.841, 3.285); p < 0.001]

Drug history

- % maximal thiazolidinedione dose [OR 1.009 (95% CI 0.999, 1.020); p = 0.074]
- baseline peripheral vasodilator therapy [OR 1.792 (95% CI 1.039, 3.090); p = 0.036]
- baseline calcium channel blocker therapy [OR 1.506 (95% CI 1.146, 1.979); p = 0.003]
- baseline diltiazem therapy [OR 2.030 (95% CI 1.335, 3.088); p = 0.001]
- baseline beta blocker therapy [OR 1.279 (95% CI 0.975, 1.679); p = 0.076]
- baseline central antihypertensive therapy [OR 2.374 (95% CI 0.933, 6.042); p = 0.070]
- baseline nitrates [OR 2.238, (95% CI 1.681, 2.979); p < 0.001]

Clinical measurements

- baseline systolic blood pressure in mmHg [OR 1.014 (95% CI 1.004, 1.024); p = 0.007]

- baseline BMI in kg/m^2 [OR 1.053 (95% CI 1.026, 1.080); $p < 0.001$]

Laboratory-based clinical investigations

- baseline haematocrit expressed as % value [OR 0.930 (95% CI 0.895, 0.966); $p < 0.001$]
- baseline estimated glomerular filration rate in mls/min/$1.73m^2$ *(log_e transformed data)* [OR 0.422 (95% CI 0.285, 0.627); $p < 0.001$]
- baseline serum creatinine > 130 μmol/L [OR 1.993 (95% CI 1.056, 3.761); $p = 0.033$]
- baseline serum albumin in g/L *(log_e transformed data)* [OR 0.146 (95% CI 0.078, 0.274); $p < 0.001$]
- baseline alanine aminotransferase in IU/L *(log_e transformed data)* [OR 0647 (95% CI 0.474, 0.883); $p = 0.006$]

Echocardiographic parameters

- baseline left ventricular mass > 228 g [OR 6.522 (95% CI 1.429, 29.766); $p = 0.015$]
- baseline interventricular septal width in cm [OR 6.485 (95% CI 1.178, 35.694); $p = 0.032$]

A detailed description of Odd's ratios for each individual covariate, with their 95% CI, are given in tables 3.42 and 3.43 respectively.

Table 3.42 - Univariate logistic regression analysis: baseline continuous independent variables predicting index loop diuretic prescription within one year of inclusion into the metformin-sulphonylurea or thiazolidinedione cohort.

Baseline continuous variable	*N (index loop diuretics prescribed [patients with variable data])*	*B*	*SE*	*Wald*	*df*	*p*	*OR [Exp (B)]*	*Lower 95% CI for Exp (B)*	*Upper 95% CI for Exp (B)*	*NR^2*	*H-L statistic*
Age (years)	221 (4882)	0.046	0.007	43.436	1	< 0.001	1.047	1.033	1.061	0.030	0.841
Diabetes duration (years)[a]	221(4882)	0.255	0.067	14.435	1	< 0.001	1.290	1.131	1.472	0.010	0.457
MAP (mmHg)	180 (4283)	0.006	0.609	0.465	1	0.495	1.006	0.989	1.023	0.000	0.466
SBP (mmHg)	180 (4283)	0.014	0.005	7.400	1	0.007	1.014	1.004	1.024	0.006	0.419
DBP (mmHg)	180 (4283)	- 0.012	0.009	1.649	1	0.199	0.988	0.971	1.006	0.001	0.351
Weight (kg)	193 (4453)	0.004	0.004	1.090	1	0.296	1.005	0.996	1.013	0.001	0.737
BMI (kg/m^2)	183 (4453)	0.052	0.013	15.585	1	< 0.001	1.053	1.026	1.080	0.012	0.001
Haematocrit (%)	158 (3579)	- 0.073	0.020	13.678	1	< 0.001	0.930	0.895	0.966	0.012	0.184
Baseline HbA1c (%)	199 (4538)	- 0.047	0.051	0.881	1	0.348	0.954	0.864	1.053	0.001	0.402
Total cholesterol (mmol/L)[b]	181 (4388)	- 0.208	0.332	0.393	1	0.531	0.812	0.424	1.557	0.000	0.546
HDL-C (mmol/L)[b]	153 (3953)	0.334	0.324	1.061	1	0.303	1.396	0.740	2.636	0.001	0.001
LDL-C (mmol/L)[a]	111 (2973)	- 0.558	0.308	3.289	1	0.070	0.572	0.313	1.046	0.004	0.497
Trigs (mmol/L)[b]	139 (3419)	- 0.014	0.153	0.009	1	0.926	0.986	0.731	1.330	0.000	0.494
ALT (IU/L)[b]	170 (4010)	- 0.435	0.159	7.524	1	0.006	0.647	0.474	0.883	0.006	0.852
Sodium (mmol/L)	193 (4469)	- 0.037	0.026	2.027	1	0.154	0.964	0.916	1.014	0.002	0.565
eGFR ($mls/min/1.73m^2$)[b]	160 (3995)	- 0.862	0.201	18.358	1	< 0.001	0.422	0.285	0.627	0.016	0.067
TSH (mIU/L)[a]	173 (3778)	0.119	0.171	0.485	1	0.486	1.126	0.806	1.574	0.000	0.056

Baseline continuous variable	***N*** ***(index loop diuretics prescribed [patients with variable data])***	***B***	***SE***	***Wald***	***df***	***p***	***OR [Exp (B)]***	***Lower 95% CI for Exp (B)***	***Upper 95% CI for Exp (B)***	***NR^2***	***H-L statistic***
Serum albumin (g/L)[b]	183 (4203)	- 1.921	0.319	36.197	1	< 0.001	0.146	0.078	0.274	0.029	0.915
TZD dose (% maximal)	90 (2097)	0.009	0.005	3.198	1	0.074	1.009	0.999	1.020	0.005	0.023
IVS (cm)	15 (291)	1.869	0.870	4.616	1	0.032	6.485	1.178	35.694	0.044	0.901
LVPW (cm)[b]	14 (260)	1.751	1.070	2.677	1	0.102	5.760	0.707	46.911	0.027	0.344

[a] square root transformed; [b] log_e transformed; [c] reciprocally transformed; ALT, alanine aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated haemoglobin; HDL-C, high density lipoprotein cholesterol; IVS, interventricular septum width; LDL-C, low density lipoprotein cholesterol; LVM, left ventricular mass; LVPW, left ventricular posterior wall thickness; MAP, mean arterial pressure; SBP, systolic blood pressure; trigs, triglycerides; TSH, thyroid stimulating hormone; TZD thiazolidinedione.

Table 3.43 - Univariate logistic regression analysis: baseline categorical independent variables predicting index loop diuretic prescription within one year of inclusion into the metformin-sulphonylurea or thiazolidinedione cohort

Baseline categorical variable	*N (categorical variable of interest [patients with variable data])*	*N (categorical variable loop diuretic +ve [categorical variable loop diuretic –ve])*	*B*	*SE*	*Wald*	*df*	*p*	*OR [Exp (B)]*	*Lower 95% CI for Exp (B)*	*Upper 95% CI for Exp (B)*	*NR^2*
Male gender	2898 (4882)	114 (2784)	- 0.331	0.138	5.758	1	0.016	0.718	0.548	0.941	0.004
Female gender	1984 (4882)	107 (1877)	0.331	0.138	5.758	1	0.016	1.392	1.062	1.824	0.004
TZD + insulin	70 (2097)	5 (65)	0.564	0.477	1.397	1	0.237	1.757	0.690	4.477	0.002
TZD (vs MFSU)	2097 (4882)	90 (2007)	- 0.096	0.140	0.469	1	0.493	0.908	0.690	1.196	0.000
Creat > 130 μmol/L	183 (4203)	11 (172)	0.690	0.324	4.529	1	0.033	1.993	1.056	3.761	0.003
Peripheral vasodilator	197 (4882)	15 (182)	0.583	0.278	4.405	1	0.036	1.792	1.039	3.090	0.003
Thiazide diuretic	1534 (4882)	77 (1457)	0.162	0.145	1.254	1	0.263	1.176	0.886	1.561	0.001
Potassium sp. diuretic	71 (4882)	5 (66)	0.477	0.469	1.035	1	0.309	1.612	0.643	4.041	0.001
NSAID	3302 (4882)	155 (3147)	0.122	0.150	0.660	1	0.417	1.130	0.842	1.517	0.000
Dihydropyridine CCB	1650 (4882)	95 (1555)	0.409	0.139	8.630	1	0.003	1.506	1.146	1.979	0.006
Verapamil	56 (4882)	4 (52)	0.491	0.524	0.879	1	0.348	1.634	0.586	4.558	0.001
Diltiazem	326 (4882)	27 (299)	0.708	0.214	10.960	1	0.001	2.030	1.335	3.088	0.006
Beta blockers	1887 (4882)	98 (1789)	0.246	0.139	3.149	1	0.076	1.279	0.975	1.679	0.002
Vasodilat	25 (4882)	2 (23)	0.611	0.740	0.680	1	0.410	1.842	0.431	7.861	0.000
Caanitht	50 (4882)	5 (45)	0.865	0.477	3.293	1	0.070	2.374	0.933	6.042	0.002
Anbd	9 (4882)	1 (8)	0.972	1.063	0.837	1	0.360	2.644	0.329	21.231	0.000

Baseline categorical variable	***N*** ***[categorical variable of interest (patients with variable data)]***	***N*** ***[categorical variable loop diuretic +ve (categorical variable loop diuretic -ve)]***	***B***	***SE***	***Wald***	***df***	***p***	***OR*** ***[Exp (B)]***	***Lower 95% CI for Exp (B)***	***Upper 95% CI for Exp (B)***	***NR^2***
Aabd	366 (4882)	21 (345)	0.273	0.236	1.334	1	0.248	1.314	0.827	2.087	0.001
ACEI	2191 (4882)	103 (2088)	0.073	0.138	0.279	1	0.597	1.076	0.821	1.410	0.000
ARB	539 (4882)	28 (511)	0.164	0.208	0.624	1	0.429	1.178	0.784	1.770	0.000
Nitrates	976 (4882)	77 (899)	0.805	0.146	30.441	1	<0.001	2.238	1.681	2.979	0.018
Otherantiang	97 (4882)	2 (95)	- 0.823	0.718	1.316	1	0.251	0.439	0.107	1.792	0.001
Macrovascular disease	866 (4882)	74 (792)	0.900	0.148	37.078	1	< 0.001	2.459	1.841	3.285	0.022
LVM > 228g	14 (254)	12 (2)	1.875	0.775	5.860	1	0.015	6.522	1.429	29.766	0.093

Aabd, alpha adrenoceptor drugs; ACEI, angiotensin converting enzyme inhibitors; Anbd, adrenergic neurone blocking drugs; ARB, angiotensin II receptor antagonists; Caantiht, centrally acting antihypertensive drugs; Dihydropyridine CCB, dihydropyridine calcium channel blockers; Creat, serum creatinine; LVM, left ventricular mass; MFSU, metformin-sulphonylurea combination therapy; NSAID, non-steroidal anti-inflammatory drug; otherantiang, other antianginal drugs; Potassium sp. diuretic, potassium sparing diuretic therapy; trigs, triglycerides; TZD, thiazolidinedione; TZD + insulin, thiazolidinedione-insulin combination therapy; Vasodilat, vasodilator antihypertensive drugs;

3.12.2 Multivariate logistic regression

90 and 131 patients required prescription of an index loop diuretic within one year after exposure to thiazolidinedione therapy and metformin-sulphonylurea combination therapy respectively. 2007 thiazolidinedione-treated patients and 2654 patients on metformin-sulphonylurea combination therapy did not develop require an index loop diuretic after inclusion into their respective cohort. Hence, the overall proportion of patients requiring an index loop diuretic prescription amounts to 0.04526 (or 4.53%).

Based on statistical work reported by Peduzzi et al. [609], given the proportion of patients requiring an index loop diuretic after index thiazolidinedione prescription, the maximum number of covariates that can be included in any model amounts to 22.

Based on univariate analysis, and taking into account the number of patients for whom data for each covariate were available, covariates of interest were modelled into two stepwise index loop diuretic logistic regression models (1 and 2).

(i) Index loop diuretic logistic regession model 1

The following predictors (covariates) were included in index loop diuretic logistic regression model 1

- Age (years)
- diabetes duration (years) *(square root transformed data)*

- baseline BMI (kg/m^2)
- baseline haematocrit (%)
- baseline serum creatinine > 130 μmol/L
- baseline albumin (g/L) *(log_e transformed data)*
- baseline ALT (IU/L) *(log_e transformed data)*
- baseline systolic blood pressure (mmHg)
- female gender
- baseline macrovascular disease (composite of coronary artery disease, peripheral artery disease and cerebrovascular disease)
- index thiazolidinedione prescription (vs baseline metformin-sulphonylurea combination therapy)

% maximal thiazolidinedione dose was not included into the logistic regression model, so as not to restrict the model to thiazolidinedione-treated patients. Index thiazolidinedione prescription (vs metformin-sulphonylurea combination therapy) was included as a covariate despite not reaching statistical significance on univariate regression, given this study's aim of investigating whether PPAR-γ agonist therapy predicts index loop diuretic prescription in a multivariate model.

3116 patients were included into the logistic regression model. Employing a 0.05 criterion of statistical significance, the Wald criterion demonstrated that baseline BMI, baseline age, baseline macrovascular disease, baseline serum albumin and diabetes duration made a significant contribution to prediction, as shown in table 3.44.

A test of the full model versus a model with intercept only was statistically significant (chi square 82.198, $p < 0.001$ with df = 5). The p value for the Hosmer and Leneshow test statistic (H-L statistic) was greater than 0.05 (chi square 6.761, df = 8, p = 0.563), implying that the model's estimates fit the data at an acceptable level. Nagelkerke's $R^2 = 0.091$, effectively indicating a relationship of 9.1% between predictors (covariates) and the prediction (ie index loop diuretic prescription). Prediction success overall was 96.0%.

Wald's statistic for the final model indicate that baseline BMI, age and baseline macrovascular disease are the strongest predictors of fluid overload (in decreasing order of importance). From table 3.44, the fitted model is:

Logit (p) = - 7.413 + (0.085*BMI) + (0.053*age) + (0.723*macrovascular disease) + (-1.339*serum albumin *[$\log_e$ transformed data]*) + (0.214*diabetes duration *[square root transformed data]*)

where p is the probability of progessing to index loop diuretic prescription within one year.

Thus, when holding all other variables constant, a patient known to suffer from macrovascular disease at metformin-sulphonylurea combination or thiazolidinedione prescription is 2.06 times more likely to require prescription of an index loop diuretic within one year after inclusion into either cohort. With each unit square root (√) passing year since diagnosis of T2DM, a patient's risk of requiring index loop diuretic prescription after inclusion into either cohort increases by 23.9%, assuming all other covariates are unchanged during the observation period. Holding all other variables constant, each one year increase in age at prescription of metformin-

sulphonylurea combination or thiazolidinedione therapy is associated with a 5.5% increased risk of fluid overload. Each 1 kg/m^2 increase in baseline BMI is likewise associated with an 8.8% increased risk of index loop diuretic prescription, assuming all other covariates are held constant. Inverting odd's ratios and holding all other variables constant, T2DM patients treated with metformin-sulphonylurea combination therapy/thiazolidinediones are at 3.82 times increased risk of fluid overload per g/L reduction in baseline $\log_e$ serum albumin. Index thiazolidinedione therapy did not contribute as a covariate in both the final model, as was observed in univariate regression, suggesting that any thiazolidinedione-associated index loop diuretic prescription (acting as a surrogate marker of fluid retention) is accounted for by other predisposing factors.

ROC curve analysis was used to discriminate between positive and negative cases. Concardance index (c-statistic/AUC) for this model amounted to 0.713 (95% CI 0.673, 0.753) ($p < 0.001$), suggesting that the final model has an ability to distinguish between the two outcome groups

Table 3.44 - Index loop diuretic logistic regression model 1: final model covariates predicting index loop diuretic prescription within one year of inclusion into the metformin-sulphonylurea combination or thiazolidinedione cohort*

Final model covariates	*B*	*SE*	*Wald*	*df*	*p*	*OR [exp (B)]*	*95% CI lower*	*95% CI upper*
Baseline body mass index (kg/m^2)	0.085	0.018	23.204	1	< 0.001	1.088	1.052	1.127
Age (years)	0.053	0.011	22.043	1	< 0.001	1.055	1.032	1.078
Baseline macrovascular disease	0.723	0.195	13.727	1	< 0.001	2.061	1.406	3.021
Baseline serum albumin (g/L)[a]	-1.339	0.420	10.176	1	0.001	0.262	0.115	0.597
Diabetes duration (years)[b]	0.214	0.096	4.982	1	0.026	1.239	1.026	1.495
Constant	-7.413	1.514	23.973	1	< 0.001	0.001		

**Baseline covariates included in the model were age, diabetes duration [b], body mass index, haematocrit, serum creatinine > 130 µmol/L, serum albumin [a], alanine aminotranferase [a], systolic blood pressure, female gender, macrovascular disease, thiazolidinedione therapy (vs metformin-sulphonylurea combination therapy); [a] log_e transformed data; [b]square root transformed data;*

(ii) Index loop diuretic logistic regression model 2

In order to model for baseline drug therapy, a binary logistic regression model was run with age, diabetes duration *(square root transformed data)*, baseline clinical variables (BMI, systolic blood pressure, haematocrit, serum creatinine > 130 µmol/L, serum albumin *(log_e transformed data)*, alanine aminotransferase *(log_e transformed data)*, female gender, baseline drug therapy (dihydropyridine calcium channel blockers, diltiazem, beta blockers, nitrates) and baseline index thiazolidinedione prescription (vs metformin-sulphonylurea combination therapy). Essentially, these covariates are identical to those included in logistic regression step 1, save baseline macrovascular disease, with the addition of the baseline drugs referred to above. 3116 patients were fitted

into the model, with age, diabetes duration, baseline BMI, baseline serum albumin and baseline nitrate therapy predicting index loop diuretic prescription within one year of index thiazolidinedione / metformin-sulphonylurea combination therapy (table 3.45). Thus baseline nitrate therapy carries an 84.3% increased risk of progressing to index loop diuretic therapy within one year, provided all other covariates are held constant.

Table 3.45 - Index loop diuretic logistic regression model 2: final model covariates predicting index loop diuretic prescription within one year of inclusion into the metformin-sulphonylurea combination or thiazolidinedione cohort*

Final model covariates	*B*	*SE*	*Wald*	*df*	*p*	*OR [exp (B)]*	*95% CI lower*	*95% CI upper*
Age (years)	0.054	0.011	23.219	1	< 0.001	1.055	1.032	1.078
Baseline body mass index (kg/m^2)	0.080	0.018	20.678	1	< 0.001	1.083	1.046	1.121
Baseline serum albumin (g/L)[a]	- 1.506	0.420	12.857	1	< 0.001	0.222	0.097	0.505
Baseline nitrate	0.611	0.193	10.079	1	0.002	1.843	1.263	2.687
Diabetes duration (years)[b]	0.214	0.096	4.951	1	0.026	1.238	1.026	1.494
Constant	- 6.900	1.501	21.133	1	< 0.001	0.001		

**Baseline covariates included in the model were age, diabetes duration, body mass index, haematocrit, serum creatinine, serum albumin, alanine aminotranferase, systolic blood pressure, female gender, calcium channel blockers, diltiazem, beta-blockers, nitrates and thiazolidinedione therapy (vs metformin-sulphonylurea combination therapy).* [a] *log_e transformed data;* [b] *square root transformed data.*

Model chi square = 78.862, p < 0.001 with df = 5; NR^2 = 0.087; H-L statistic chi square = 6.183, p = 0.627 with df = 8; prediction success overall = 96.0 %; ROC (AUC) = 0.711 (95% CI 0.670, 0.752), p < 0.001

3.13 Cox regression model: predicting risk factors for index loop diuretic prescription required within one year after index metformin-sulphonylurea combination or thiazolidinedione therapy

3.13.1 Univariate Cox regression

Following on from the results of logistic regression analysis, this study sought to model onset time to index loop diuretic prescription (the 'failure event') following index prescription to metformin-sulphonylurea combination therapy or thiazolidinediones, using Cox proportional hazards regression analysis.

On univariate analysis (tables 3.46 and 3.47) the following clinical and pathological factors were associated with time to index loop diuretic prescription:

Demographics

- age in years [HR 1.046 (95% CI 1.032, 1.059); $p < 0.001$]
- diabetes duration in years *(square root transformed data)* [HR 1.294 (95% CI 1.138, 1.472); $p < 0.001$]
- female gender ($p = 0.011$)

Past medical history

- baseline macrovascular disease ($p < 0.001$)

Drug history

- baseline peripheral vasodilator therapy ($p = 0.031$)

Table 3.46 - Univariate Cox regression: baseline continuous independent variable predicting index loop diuretic prescription within one year of inclusion into the metformin-sulphonylurea or thiazolidinedione cohort

Baseline continuous variable	*N [index loop diuretics prescribed (patients with variable data)]*	*B*	*SE*	*Wald*	*df*	*p*	*Hazard ratio [Exp (B)]*	*Lower 95% CI for Exp (B)*	*Upper 95% CI for Exp (B)*	*- 2 Log Likelihood*
Age	221 (4882)	0.045	0.007	44.290	1	<0.001	1.046	1.032	1.059	3660.894
Diabetes duration (years) [a]	221 (4882)	0.258	0.066	15.392	1	<0.001	1.294	1.138	1.472	3690.840
MAP (mmHg)	180 (4283)	0.005	0.008	0.387	1	0.534	1.005	0.989	1.022	2970.030
SBP (mmHg)	180 (4283)	0.013	0.005	7.194	1	0.007	1.013	1.004	1.023	2963.439
DBP (mmHg)	180 (4283)	- 0.012	0.009	1.829	1	0.176	0.988	0.970	1.006	2968.585
Weight (kg)	183 (4453)	0.004	0.004	1.017	1	0.313	1.004	0.996	1.013	3034.441
BMI (kg/m^2)	183 (4453)	0.050	0.013	15.601	1	<0.001	1.052	1.026	1.078	3020.699
Haematocrit (%)	158 (3579)	- 0.074	0.019	15.447	1	<0.001	0.929	0.895	0.964	2532.968
HbA1c (%)	199 (4538)	- 0.040	0.050	0.632	1	0.427	0.961	0.872	1.060	3306.628
Total cholesterol (mmol/L) [b]	181 (4388)	- 0.264	0.327	0.654	1	0.419	0.768	0.404	1.457	2993.218
HDL-C (mmol/L) [b]	153 (3953)	0.319	0.321	0.991	1	0.320	1.376	0.734	2.581	2498.555
LDL-C (mmol/L) [a]	111 (2973)	- 0.571	0.301	3.589	1	0.058	0.565	0.313	1.020	1745.692
Trigs (mmol/L) [b]	139 (3419)	- 0.033	0.150	0.048	1	0.827	0.968	0.721	1.299	2229.738
ALT (IU/L) [b]	170 (4010)	- 0.448	0.156	8.208	1	0.004	0.639	0.471	0.868	2772.766
Sodium (mmol/L)	193 (4469)	- 0.038	0.026	2.149	1	0.143	0.963	0.916	1.013	3198.839
eGFR ($mls/min/1.73m^2$) [b]	160 (3995)	- 0.859	0.198	18.790	1	<0.001	0.423	0.287	0.624	2599.658
TSH (mIU/L) [a]	173 (3778)	0.121	0.168	0.512	1	0.474	1.128	0.811	1.569	2810.337
Serum albumin (g/L) [b]	183 (4203)	-1.910	0.312	37.396	1	< 0.001	0.148	0.080	0.273	2974.080

Baseline continuous variable	***N*** ***(index loop diuretics prescribed [patients with variable data])***	***B***	***SE***	***Wald***	***df***	***p***	***Hazard ratio*** ***(Exp [B])])***	***Lower 95% CI for Exp (B)***	***Upper 95% CI for Exp (B)***	***- 2 Log Likelihood***
TZD dose (% maximal)	90 (2007)	0.007	0.005	1.915	1	0.166	1.007	0.997	1.017	1354.430
IVS (cm)	15 (291)	1.948	0.838	5.397	1	0.020	7.014	1.356	36.280	162.385
LVPW (cm) [b]	14 (260)	1.538	0.874	3.092	1	0.079	4.653	0.838	25.826	150.408

ALT, alanine aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated haemoglobin; HDL-C, high density lipoprotein cholesterol; IVS, interventricular septum width; LDL-C, low density lipoprotein cholesterol; LVM, left ventricular mass; LVPW, left ventricular posterior wall thickness; MAP, mean arterial pressure; SBP, systolic blood pressure; trigs, triglycerides; TSH, thyroid stimulating hormone; TZD thiazolidinedione; [a] square root transformed data; [b] log_e transformed data

Table 3.47 - Univariate Cox regression analysis (Kaplan-Meier survival): baseline categorical independent variables predicting index loop diuretic prescription within one year of inclusion into the metformin-sulphonylurea or thiazolidinedione cohort.

			Categorical variable of interest				*Comparator categorical variable*				*Log rank test*		
Baseline categorical variable	***N [categorical variable of interest (patients with variable data)]***	***N [categorical comparator variable loop diuretic +ve (patients with comparator variable data)]***	***Mean Survival time***	***SE Survival time***	***Lower 95% CI for survival time***	***Upper 95% CI for survival time***	***Mean Survival time***	***SE Survival time***	***Lower 95% CI for survival time***	***Upper 95% CI for survival time***	***Chi Square***	***df***	***p***
Male gender	2898 (4882)	114 (2784)	357.032	0.815	355.434	358.631	354.485	1.118	352.293	356.677	6.394	1	0.011
Female gender	1984 (4882)	107 (1877)	354.485	1.118	352.293	356.677	357.032	0.815	355.434	358.631	6.394	1	0.011
TZD (vs MFSU)	70 (2097)	90 (2007)	356.670	0.977	354.756	358.584	355.506	0.902	353.739	357.274	0.420	1	0.517
TZD + insulin	2097 (4882)	5 (65)	347.677	7.624	332.734	362.620	356.970	0.975	355.058	358.882	2.489	1	0.115
Creat > 130µmol/L	183 (4203)	11 (172)	344.951	5.855	333.476	356.426	356.990	0.678	355.661	358.320	5.421	1	0.020
Peripheral vasodilators	197 (4882)	15 (182)	350.529	3.955	342.777	358.281	356.232	0.671	354.917	357.548	4.661	1	0.031
Thiazide diuretics	1534 (4882)	77 (1457)	354.998	1.248	352.553	357.444	356.463	0.781	354.932	357.994	1.486	1	0.223
Potassium sp. diuretics	71 (4882)	5 (66)	354.448	6.044	342.602	366.293	356.028	0.668	354.719	357.336	1.094	1	0.296
NSAIDs	3302 (4882)	155 (3147)	355.927	0.808	354.344	357.510	356.157	1.167	353.871	358.444	0.752	1	0.386
Dihydropyridine CCBs	1650 (4882)	95 (1555)	352.620	1.344	349.986	355.255	357.736	0.729	356.307	359.164	9.189	1	0.002
Verapamil	56 (4882)	4 (52)	352.502	6.231	340.289	364.716	356.049	0.667	354.740	357.357	0.769	1	0.380
Diltiazem	326 (4882)	27 (299)	348.725	3.414	342.034	355.416	356.524	0.668	355.216	357.832	11.764	1	0.001
Beta blockers	1887 (4882)	98 (1789)	353.968	1.191	351.634	356.303	357.299	0.778	355.775	358.824	3.392	1	0.066
Vasodilat	25 (4882)	2 (23)	342.760	15.236	312.897	372.623	356.072	0.663	354.774	357.371	0.583	1	0.445
Caanitht	50 (4882)	5 (45)	346.209	9.536	327.519	364.899	356.106	0.663	354.808	357.406	3.495	1	0.062

			Categorical variable of interest				*Comparator categorical variable*				*Log rank test*		
Baseline categorical variable	***N (categorical variable of interest [patients with categorical variable data])***	***N (categorical comparator variable loop diuretic +ve [patients with comparator variable data])***	***Mean Survival time***	***SE Survival time***	***Lower 95% CI for survival time***	***Upper 95% CI for survival time***	***Mean Survival time***	***SE Survival time***	***Lower 95% CI for survival time***	***Upper 95% CI for survival time***	***Chi Square***	***df***	***p***
Anbd	9 (4882)	1 (8)	327.778	35.093	258.995	396.561	356.057	0.662	354.760	357.354	1.031	1	0.310
Aabd	366 (4882)	21 (345)	353.674	2.594	348.590	358.759	356.180	0.687	354.834	357.526	1.747	1	0.186
ACEI	2191 (4882)	103 (2088)	356.013	0.964	354.124	357.901	355.992	0.914	354.200	357.784	0.401	1	0.527
ARB	539 (4882)	28 (54)	354.076	2.208	349.749	358.403	356.242	0.694	354.882	357.603	0.854	1	0.355
Nitrates	976 (4882)	77 (899)	349.032	1.933	345.244	352.819	357.742	0.672	356.425	359.059	33.074	1	<0.001
Otherantiang	97 (4882)	2 (95)	360.144	3.694	352.904	367.385	355.920	0.673	354.601	357.240	1.365	1	0.243
Macrovascular disease	866 (4882)	74 (792)	347.749	2.135	343.565	351.932	357.784	0.660	356.491	359.076	40.515	1	<0.001
LVM > 228g	14 (254)	12 (2)	341.470	6.917	327.913	355.027	362.222	2.145	358.017	366.427	7.513	1	0.006

aabd, alpha adrenoceptor drugs; ACEI, angiotensin converting enzyme inhibitors; anbd, adrenergic neurone blocking drugs; ARB, angiotensin II receptor antagonists; caantiht, centrally acting antihypertensive drugs; CAD, coronary artery disease; Ccb, calcium channel blockers; creat, serum creatinine; Ks, potassium sparing diuretic therapy; macrovasc, macrovascular disease; NSAID, non-steroidal anti-inflammatory drug; otherantiang, other antianginal drugs; trigs, triglycerides; TZD + insulin, thiazolidinedione-insulin combination therapy; vasodilat, vasodilator antihypertensive drugs;

- baseline dihydropyridine calcium channel blocker therapy (p = 0.002)
- baseline diltiazem therapy (p = 0.001)
- baseline beta blocker therapy (p = 0.066)
- baseline central antihypertensive therapy (p = 0.062)
- baseline nitrates (p < 0.001)

Clinical measurements

- baseline systolic blood pressure in mmHg [HR 1.013 (95% CI 1.004, 1.023); p = 0.007]
- baseline BMI in kg/m^2 [HR 1.052 (95% CI 1.026, 1.078); p < 0.001]

Laboratory-based clinical investigations

- baseline haematocrit expressed as % value [HR 0.929 (95% CI 0.895, 0.964)]; p < 0.001]
- baseline estimated glomerular filration rate in mls/min/1.73m^2 *(log_e transformed data)* [HR 0.423 (95% CI 0.287, 0.624); p < 0.001]
- baseline serum creatinine >130 µmol/L (p = 0.020)
- baseline serum albumin in g/L *(log_e transformed data)* [HR 0.148 (95% CI 0.080, 0.273); p < 0.001]
- baseline LDL-cholesterol in mmol/L *(square root transformed data)* [HR 0.565 (95% CI 0.313, 1.020); p = 0.058]
- baseline alanine aminotransferase in IU/L *(loge transformed data)* [HR 0.639 (95% CI 0.471, 0.868); p = 0.004]

Echocardiographic parameters

- baseline left ventricular mass > 228g (p = 0.006)
- baseline interventricular septal width in cm [HR 7.014 (95% CI 1.356, 36.280); p = 0.020]
- baseline left ventricular posterior wall thickness in cm *(log_e transformed data)* [HR 4.653 (95% CI 0.838, 25.826); p = 0.079]

Thus, neither baseline thiazolidinedione therapy nor % maximal thiazolidinedione dose were associated with time to progression to fluid overload.

3.13.2 Multivariate Cox regression

(i) Loop diuretic Cox regression model 1

Based on the outcomes of univariate analysis, Cox regression was used to assess the strength of association between time to index loop diuretic prescription and clinical and pathological risk factors. As outlined in multivariate logistic regression analysis, the maximum number of covariates that could be included in Cox regression analysis, based on the available data, amounted to 22.

Variables (covariates) included in multivariate Cox regression analysis were those deemed significant (p < 0.1) on univariate screening (tables 3.46 and 3.47), namely:

- Age (years)
- Female gender
- Diabetes duration (years) *(square root transformed data)*

- Baseline BMI (kg/m^2)
- Baseline systolic blood pressure (mmHg)
- Baseline haematocrit (%)
- Baseline serum creatinine > 130 μmol/L
- Baseline serum albumin (g/L) *(log_e transformed data)*
- Baseline alanine aminotransferase (IU/L) *(log_e transformed data)*
- Baseline macrovascular disease
- Index thiazolidinedione prescription (vs metformin-sulphonylurea)

Given that female gender, baseline serum creatinine > 130 μmol/L, macrovascular disease and index thiazolidinedione prescription defied the Proportional Hazards Assumption, time-dependent variables were constructed for each variable by adding an interaction term that involved log_e time (days) to index loop diuretic prescription into the Cox model, and testing for its significance. Time-dependent variables were also constructed in the same fashion for age, diabetes duration, BMI, systolic blood pressure, haematocrit, serum albumin *(log_e transformed data)* and alanine aminotransferase *(log_e transformed data)* as evidence that hazard ratios for these covariates do not change over time.

There were no significant interactions between any of the included covariates in this model. Out of a total of 3116 patients, for whom data were available for this model, 126 patients required an index loop diuretic within one year of prescription of metformin-sulphonylurea combination or thiazolidinedione therapy. 2990 patients were censored within the aforementioned period of observation. The covariates as a set reliably improved the predictability of the Cox regression model (chi square

2517.726, $p < 0.001$ with df = 15). The standard error (SE) of each variable included in the model was small, suggesting no significant multicollinearity. The Wald criterion demonstrated that (in decreasing order of importance) age, baseline haematocrit, baseline BMI, baseline alanine aminotransferase, baseline systolic blood pressure and baseline macrovascular disease, and their respective interactions with time made a significant contribution to predicting time to index loop diuretic prescription in this setting, as outlined in table 3.48. Covariate*time interactions suggested a decreasing hazard ratio over time for baseline macrovascular disease, alanine aminotransferase and serum albumin. Hazard ratios for age, BMI, systolic blood pressure, and haematocrit remained relatively (albeit not completely) stable over the period of observation, in keeping with log-minus-log plots which had suggested that each of the latter covariates satisfied the Proportiona Hazards Assumption (table 3.49, figure 3.13).

Thus, the hazard ratio for requiring an index loop diuretic at time t (HR_t) associated with baseline macrovascular disease can be summarised by the equation:

$(HR_t) = \exp (8.810 - 1.527*t)$

which at t = 180 days (ie six months, $\log_e$ of which = 5.19, amounts to exp (8.810 - 1.527*5.19) = 2.423

whereas at t = 270 days (ie 9 months, $\log_e$ of which = 5.60), equals exp (8.810-1.527*5.60) = 1.295

while at t = 365 days (ie one year, $\log_e$ of which = 5.90) amounts to exp (8.810 - 1.527*5.90) = 0.819.

It can thus be concluded that baseline macrovascular disease is indeed a strong risk factor for index loop diuretic prescription within the first nine months of therapy, but that this effect wears off over time.

Table 3.48 – Loop diuretic Cox regression model 1 predicting index loop diuretic prescription within one year of inclusion into the metformin-sulphonylurea combination or thiazolidinedione cohort.

Final baseline model covariates	*B*	*SE*	*Wald*	*df*	*p*	*Hazard ratio*	*95% CI lower*	*95% CI upper*
Age (years)	1.064	0.137	60.405	1	<0.001	2.899	2.217	3.792
Body mass index (kg/m^2)	1.142	0.197	33.449	1	<0.001	3.133	2.128	4.614
Systolic BP (mmHg)	0.313	0.075	17.467	1	<0.001	1.367	1.181	1.583
Haematocrit (%)	2.029	0.265	58.484	1	<0.001	7.610	4.524	12.802
Serum albumin (g/L) [a]	8.955	4.692	3.642	1	0.056	7746.095	0.785	76423616.57
ALT (IU/L) [a]	13.816	2.509	30.334	1	<0.001	1000699.348	7328.611	136642431.4
Macrovascular disease	8.810	2.276	14.979	1	<0.001	6698.006	77.342	580066.109
Age (years)*$\log_e$time	-0.190	0.025	57.021	1	<0.001	0.827	0.787	0.869
Body mass index (kg/m^2)*$\log_e$time	-0.199	0.037	28.725	1	<0.001	0.820	0.762	0.881
Systolic BP (mmHg)*$\log_e$time	-0.058	0.014	16.686	1	<0.001	0.943	0.918	0.970
Haematocrit (%)*$\log_e$time	-0.371	0.049	57.450	1	<0.001	0.690	0.627	0.759
Serum albumin (g/L) *$\log_e$time	-1.950	0.875	4.964	1	0.026	0.142	0.026	0.791
ALT (IU/L) *$\log_e$time	-2.595	0.471	30.330	1	<0.001	0.075	0.030	0.188
Macrovascular disease*$\log_e$time	-1.527	0.435	12.320	1	<0.001	0.217	0.093	0.510

**Baseline covariates included in the model were age, female gender, diabetes duration [b], body mass index, systolic blood pressure, haematocrit, serum creatinine > 130 µmol/L, serum albumin[a], alanine aminotranferase [a], macrovascular disease, index thiazolidinedione (vs metformin-sulphonylurea therapy)*

Events = 126, censored = 2990; - 2 LL = 683.747; Model chi square = 2679.979, p < 0.001 with df = 14

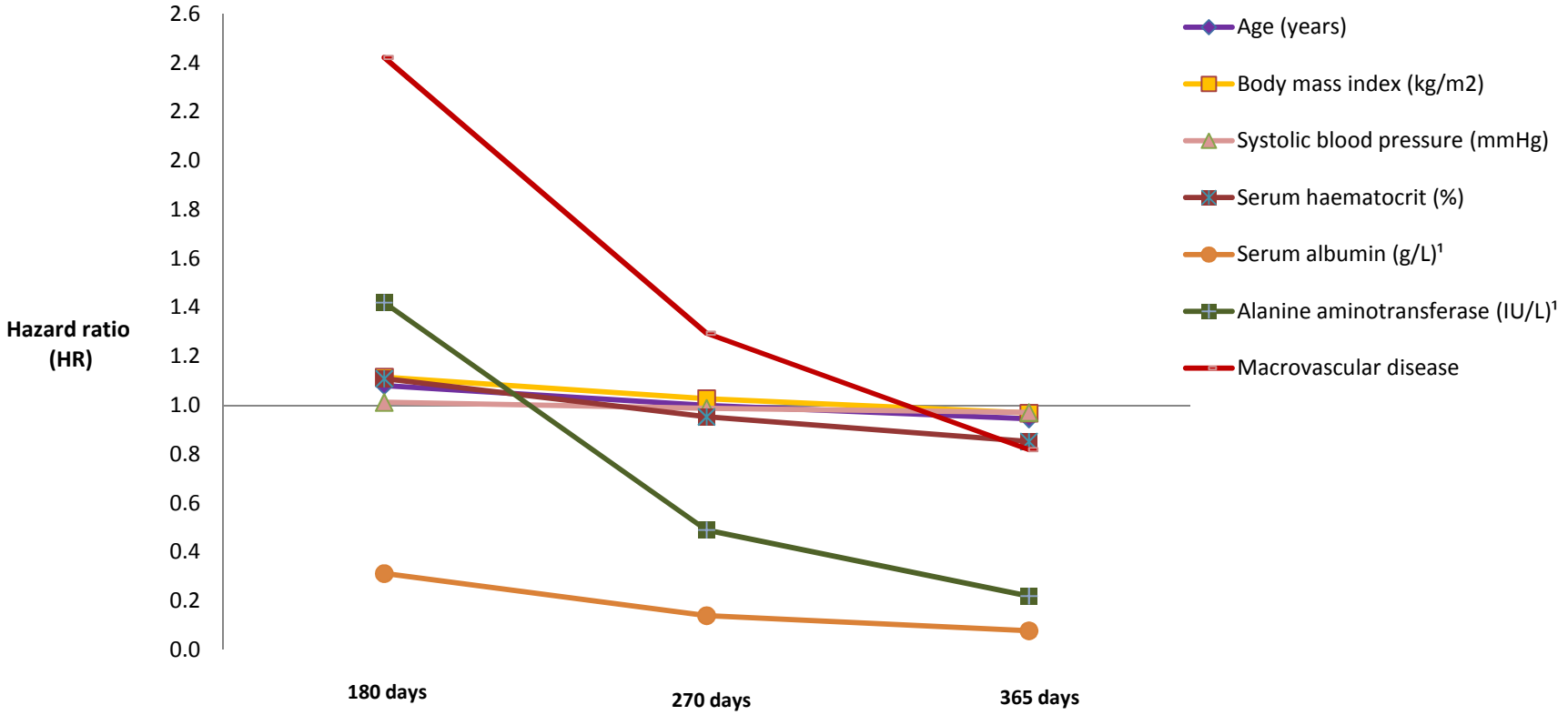
[a] Square root transformed data; [b] loge transformed data

Table 3.49 - Three monthly variation in estimated hazard ratios (HR) for index loop diuretic prescription after index metformin-sulphonylurea or thiazolidinedione prescription. HR were estimated at six months, nine months and one year for all significant covariates in loop Cox regression model 1.

Time-dependent covariates	*HR at 6 months (180 days)*	*HR at 9 months (270 days)*	*HR at 12 months (365 days)*
Age (years)	1.08	1.00	0.95
Body mass index (kg/m^2)	1.12	1.03	0.97
Systolic blood pressure (mmHg)	1.01	0.99	0.97
Haematocrit (%)	1.11	0.95	0.85
Serum albumin (g/L) [a]	0.31	0.14	0.08
Alanine aminotransferase (IU/L) [a]	1.42	0.49	0.22
Macrovascular disease	2.42	1.30	0.82

[a] *log_e transformed data*

Figure 3.13 - Variation in hazard ratio values for index loop diuretic prescription within one year of index metformin-sulphonylurea combination or thiazolidinedione prescription: loop Cox regression model 1. Data are plotted at 180, 270 and 365 days. Patients treated with metformin-sulphonylurea combination or thiazolidinedione therapy for less than 90 days were excluded.



[1] Log_e transformed data

(ii) Loop diuretic Cox regression model 2

In this model, baseline dihydropyridine calcium channel blockers, diltiazem, beta blockers and nitrates were included as covariates in lieu of baseline macrovascular disease. All other covariates included in step 1 were maintained. Baseline age, BMI, systolic blood pressure, haematocrit, serum albumin, alanine aminotransferase and nitrates emerged as significant predictors of time to index loop diuretic prescription on multivariate analysis, as shown in table 3.50 below.

Hazard ratios for serum albumin and alanine aminotransferase exhbited a time-dependent reduction over the period of observation, as outlined in loop Cox regression model 1. Varation in risk associated with age, BMI, systolic blood pressure and haematocrit was relatively mild. Hazard ratios for background nitrate therapy remained constant throughout the first year after index metfformin-sulphonylure combination or thiazolidinedione prescription (table 3.51, figure 3.14).

The relatively small number of patients with data for baseline left ventricular mass and interventricular septum width did not permit a generation of a Cox regression model incorporating these echocardiographic variables as covariates.

Table 3.50 – Loop diuretic Cox regression model 2 predicting index loop diuretic prescription within one year of inclusion into the metformin-sulphonylurea combination or thiazolidinedione cohort

Final baseline model covariates	*B*	*SE*	*Wald*	*df*	*p*	*Hazard ratio*	*95% CI lower*	*95% CI upper*
Age (years)	0.978	0.131	55.412	1	<0.001	2.660	2.056	3.442
Body mass index (kg/m^2)	0.999	0.200	24.930	1	<0.001	2.715	1.834	4.017
Systolic BP (mmHg)	0.301	0.076	15.831	1	<0.001	1.352	1.165	1.568
Haematocrit (%)	1.754	0.269	42.589	1	<0.001	5.776	3.411	9.782
Serum albumin (g/L) [a]	12.633	5.008	6.365	1	0.012	306583.759	16.755	5609800937
ALT (IU/L) [a]	13.035	2.465	27.964	1	<0.001	458286.577	3655.219	57459369.51
Nitrates	0.505	0.217	5.426	1	0.020	1.656	1.083	2.533
Age (years)*$\log_e$time	-0.175	0.024	52.179	1	<0.001	0.839	0.801	0.880
Body mass index (kg/m^2)*$\log_e$time	-0.174	0.038	21.473	1	<0.001	0.840	0.780	0.904
Systolic BP (mmHg)*$\log_e$time	-0.056	0.014	15.388	1	<0.001	0.945	0.919	0.972
Haematocrit (%)*$\log_e$time	-0.322	0.050	41.967	1	<0.001	0.725	0.658	0.799
Serum albumin (g/L) *$\log_e$time	-2.654	0.933	8.093	1	0.004	0.070	0.011	0.438
ALT (IU/L) *$\log_e$time	-2.473	0.463	28.520	1	<0.001	0.084	0.034	0.209

**Baseline covariates included in the model were age, female gender, diabetes duration [b], body mass index, systolic blood pressure, haematocrit, serum creatinine > 130 µmol/L, serum albumin, alanine aminotranferase, dihydropyridine calcium channel blockers, diltiazem, beta blockers, nitrates and index thiazolidinedione (vs metformin-sulphonylurea therapy), together with their respective loge time-dependent covariates*

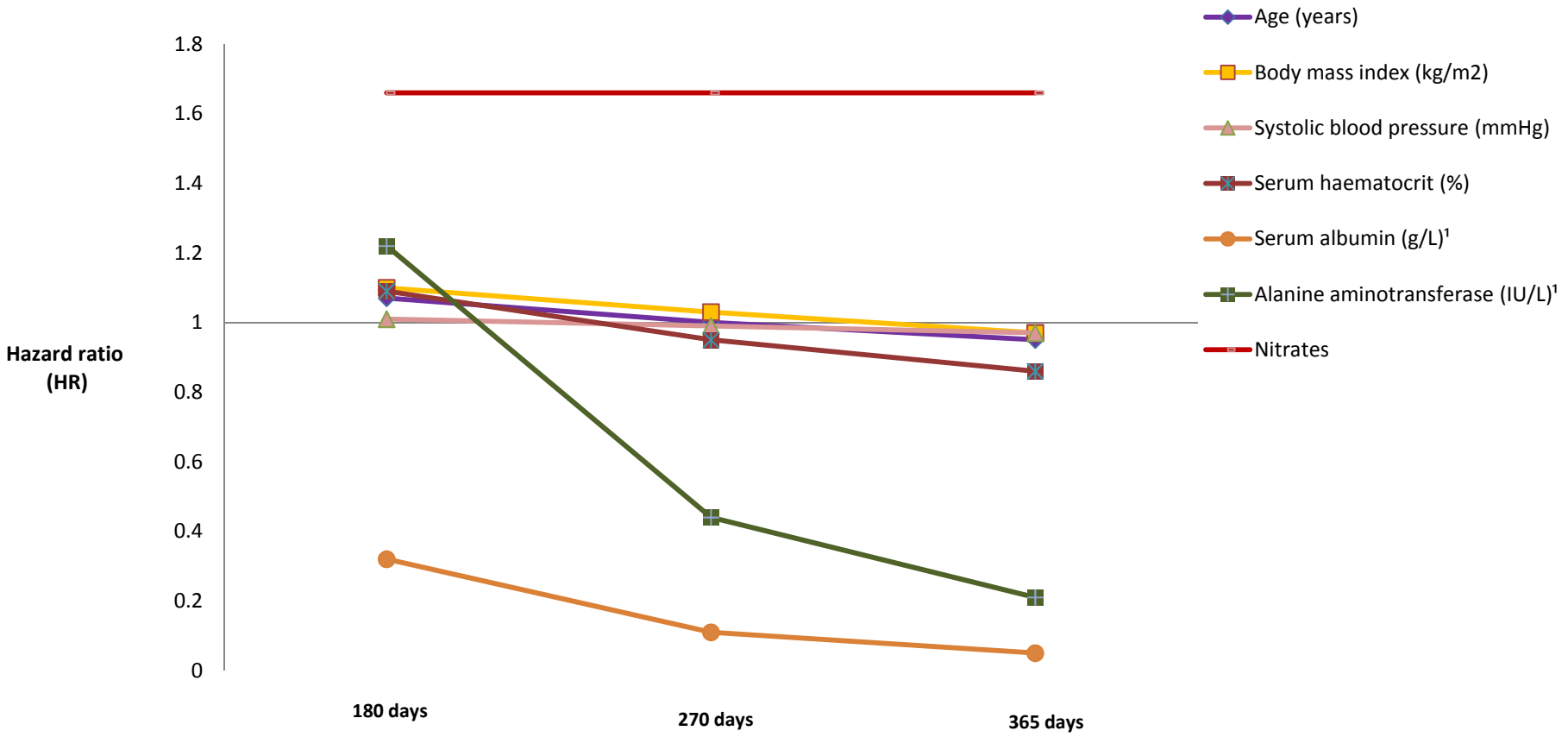
[a] loge transformed data; [b] square root transformed data; Events = 126, censored = 2990; - 2 LL = 703.617; Model chi square = 2537.137, p < 0.001 with df = 1

Table 3.51 - Three monthly variation in estimated hazard ratios (HR) for index loop diuretic prescription after index metformin-sulphonylurea or thiazolidinedione prescription. HR were estimated at six months, nine months and one year for all significant covariates in loop Cox regression model 2

Time-dependent covariates	*HR at 6 months (180 days)*	*HR at 9 months (270 days)*	*HR at 12 months (365 days)*
Age (years)	1.07	1.00	0.95
Body mass index (kg/m^2)	1.10	1.03	0.97
Systolic blood pressure (mmHg)	1.01	0.99	0.97
Haematocrit (%)	1.09	0.95	0.86
Serum albumin (g/L) [a]	0.32	0.11	0.05
Alanine aminotransferase (IU/L)[a]	1.22	0.44	0.21
Nitrates	1.66	1.66	1.66

[a] *log_e transformed data*

Figure 3.14 - Variation in hazard ratio values for index loop diuretic prescription within one year of index metformin-sulphonylurea combination or thiazolidinedione prescription: loop Cox regression model 2. Data are plotted at 180, 270 and 365 days. Patients treated with metformin-sulphonylurea combination or thiazolidinedione therapy for less than 90 days were excluded



[1] Log_e transformed data

3.14 Logistic regression model: predicting risk factors for incident heart failure events occurring within one year after index metformin-sulphonylurea combination or thiazolidinedione therapy

3.14.1 Univariate logistic regression

Univariate analysis found that incident HF occurring within one year of inclusion into either the metformin-sulphonylurea/thiazolidinedione cohort was significantly associated with the following characteristics (tables 3.52 and 3.53):

Demographics

- age in years [OR 1.064 (95% CI 1.040, 1.088); p < 0.001]
- diabetes duration in years *(square root transformed data)* [OR 1.503 (95% CI 1.209, 1.867); p < 0.001]
- female gender [OR 0.644 (95% CI 0.401, 1.036); p = 0.070]

Past medical history

- baseline macrovascular disease [OR 4.711 (95% CI 2.997, 7.405); p < 0.001]

Drug history

- baseline potassium channel blocker/aldosterone antagonist therapy [OR 3.744 (95% CI 1.902, 7.373); p < 0.001]
- baseline verapamil therapy [OR 4.470 (95% CI 1.764, 11.326); p = 0.002]
- baseline diltiazem therapy [OR 2.233 (95% CI 1.221, 4.082); p = 0.009]
- baseline beta blocker therapy [OR 2.066 (95% CI 1.307, 3.268); p = 0.002]

- baseline alpha adrenoceptor drugs [OR 1.794 (95% CI 0.963, 3.341); p = 0.066]
- baseline nitrates [OR 3.773 (95% CI 2.402, 5.928); p = 0.041]
- baseline other antianginals [OR 2.419 (95% CI 0.964, 6.066); p = 0.060]

Laboratory-based clinical investigations

- baseline haematocrit expressed as % value [OR 0.902 (95% CI 0.845, 0.962); p = 0.002]
- baseline HDL-C concentration in mmol/L *(log_e transformed data)* [OR 3.495 (95% CI 1.204, 10.146); p = 0.021]
- baseline estimated glomerular filration rate in mls/min/1.73m^2 *(log_e transformed data)* [OR 0.305 (95% CI 0.171, 0.543); p < 0.001]
- baseline serum creatinine > 130 µmol/L [OR 3.586 (95% CI 1.810, 7.104); p < 0.001]
- baseline serum albumin in g/L *(log_e transformed data)* [OR 0.135 (95% CI 0.051, 0.359); p < 0.001]
- baseline alanine aminotransferase in IU/L *(log_e transformed data)* [OR 0.428 (95% CI 0.254, 0.721); p = 0.001]

3.14.2 Multivariate logistic regression

28 and 49 patients developed incident HF within one year after exposure to thiazolidinedione therapy and metformin-sulphonylurea combination therapy respectively. 2521 thiazolidinedione-treated patients and 3427 patients on metformin-sulphonylurea combination therapy remained incident HF free within one

Table 3.52 - Univariate logistic regression analysis: baseline continuous independent variables predicting incident heart failure events within one year of inclusion into the metformin-sulphonylurea or thiazolidinedione cohort.

Baseline continuous variable	*N [index loop diuretics prescribed (patients with variable data)]*	*B*	*SE*	*Wald*	*df*	*p*	*OR (Exp [B])*	*Lower 95% CI for Exp (B)*	*Upper 95% CI for Exp (B)*	*NR^2*	*H-L statistic*
Age (years)	77 (6025)	0.062	0.012	27.911	1	< 0.001	1.064	1.040	1.088	0.038	0.850
Diabetes duration (years)[a]	77 (6025)	0407	0.111	13.509	1	< 0.001	1.503	1.209	1.867	0.017	0.414
MAP (mmHg)	66 (5302)	- 0.005	0.014	0.105	1	0.745	0.996	0.969	1.023	0.000	0.432
SBP (mmHg)	66 (5302)	0.009	0.008	1.353	1	0.245	1.009	0.994	1.026	0.002	0.961
DBP (mmHg)	66 (5302)	- 0.023	0.015	2.380	1	0.123	0.978	0.950	1.006	0.004	0.026
Weight (kg)	66 (5520)	0.004	0.007	0.331	1	0.565	1.004	0.990	1.018	0.000	0.838
BMI (kg/m^2)	66 (5520)	0.008	0.022	0.113	1	0.736	1.008	0.964	1.053	0.000	0.259
Haematocrit (%)	46 (4525)	-0.104	0.033	9.893	1	0.002	0.902	0.845	0.962	0.019	0.670
HbA1c (%)	68 (5638)	0.112	0.077	2.075	1	0.150	1.118	0.961	1.302	0.003	0.758
Total cholesterol (mmol/L)[b]	66 (5466)	0.817	0.537	2.315	1	0.128	2.265	0.790	6.491	0.003	0.367
HDL-C (mmol/L)[b]	50 (4931)	1.251	0.544	5.296	1	0.021	3.495	1.204	10.146	0.010	0.177
LDL-C (mmol/L)[a]	41 (3717)	0.370	0.491	0.569	1	0.451	1.448	0.553	3.789	0.001	0.121
Triglycerides (mmol/L)[b]	51 (4267)	- 0.392	0.260	2.271	1	0.132	0.676	0.406	1.125	0.004	0.201
ALT (IU/L)[b]	63 (5026)	- 0.848	0.266	10.184	1	0.001	0.428	0.254	0.721	0.017	0.061
Sodium (mmol/L)	69 (5571)	- 0.019	0.042	0.199	1	0.655	0.981	0.904	1.066	0.000	0.448
eGFR ($mls/min/1.73m^2$)[b]	61 (5012)	- 1.188	0.294	16.278	1	< 0.001	0.305	0.171	0.543	0.025	0.022
TSH (mIU/L)	57 (4806)	0.002	0.088	0.001	1	0.980	1.002	0.844	1.190	0.000	0.061
Serum albumin (g/L)[b]	68 (5278)	- 2.005	0.500	16.077	1	< 0.001	0.135	0.051	0.359	0.024	0.577

Baseline continuous variable	***N*** ***(index loop diuretics prescribed [patients with variable data])***	***B***	***SE***	***Wald***	***df***	***p***	***OR*** ***[Exp (B)]***	***Lower 95% CI for Exp (B)***	***Upper 95% CI for Exp (B)***	***NR^2***	***H-L statistic***
TZD dose (% maximal)[b]	28 (2549)	- 0.004	0.556	0.000	1	0.995	0.996	0.335	2.964	0.000	0.033
IVS (cm)	6 (447)	0.573	1.405	0.166	1	0.683	1.773	0.113	27.847	0.003	0.629
LVPW (cm)[b]	5 (397)	0.444	2.044	0.047	1	0.828	1.558	0.028	85.697	0.001	0.787

ALT, alanine aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated haemoglobin; Hct, haematocrit; HDL-C, high density lipoprotein cholesterol; IVS, interventricular septum width; LDL-C, low density lipoprotein cholesterol; LVM, left ventricular mass; LVPW, left ventricular posterior wall thickness; MAP, mean arterial pressure; SBP, systolic blood pressure; TSH, thyroid stimulating hormone; TZD thiazolidinedione; [a] *square root transformed data;* [b] $\log_e$ *transformed data*

Table 3.53 - Univariate logistic regression analysis: baseline categorical independent variables predicting index heart failure events within one year of inclusion into the metformin-sulphonylurea or thiazolidinedione cohort.

Baseline categorical variable	*N [categorical variable of interest (patients with variable data)]*	*N [categorical variable loop diuretic +ve (categorical variable loop diuretic –ve)]*	*B*	*SE*	*Wald*	*df*	*p*	*OR [Exp (B)*	*Lower 95% CI for Exp (B)*	*Upper 95% CI for Exp (B)*	*NR^2*
Male gender	3372 (6025)	51 (3321)	0.439	0.242	3.285	1	0.070	1.552	0.965	2.495	0.004
Female gender	2653 (6025)	26 (2627)	- 0.439	0.242	3.285	1	0.070	0.644	0.401	1.036	0.004
TZD + insulin	92 (2549)	2 (90)	0.731	0.742	0.972	1	0.324	2.078	0.486	8.889	0.003
TZD (vs MFSU)	2549 (6025)	28 (2521)	- 0.253	0.238	1.123	1	0.289	0.777	0.487	1.239	0.001
Creat >130µmol/L	68 (5278)	10 (58)	1.277	0.349	13.409	1	<0.001	3.586	1.810	7.104	0.015
Peripheral vasodilators	287 (6025)	2 (285)	- 0.635	0.719	0.780	1	0.377	0.530	0.129	2.169	0.001
Thiazide diuretics	2045 (6025)	24 (2021)	- 0.128	0.248	0.267	1	0.605	0.880	0.542	1.429	0.000
Potassium sp. diuretics	238 (6025)	10 (228)	1.320	0.346	14.588	1	<0.001	3.744	1.902	7.373	0.014
NSAIDs	4224 (6025)	52 (4172)	- 0.122	0.245	0.247	1	0.620	0.885	0.548	1.431	0.000
Dihydropyridine CCBs	2257 (6025)	34 (2223)	0.281	0.231	1.483	1	0.223	1.325	0.842	2.084	0.002
Verapamil	96 (6025)	5 (91)	1.497	0.474	9.962	1	0.002	4.470	1.764	11.326	0.009
Diltiazem	428 (6025)	13 (496)	0.803	0.308	6.809	1	0.009	2.233	1.221	4.082	0.007
Beta blockers	2532 (6025)	46 (2486)	0.726	0.234	9.633	1	0.002	2.066	1.307	3.268	0.013
Vasodilat	36 (6025)	1 (35)	0.799	1.021	0.612	1	0.434	2.223	0.301	16.435	0.001
Caanitht	94 (6025)	1 (93)	- 0.188	1.012	0.035	1	0.852	0.828	0.114	6.020	0.000
Anbd	13 (6025)	0 (13)	- 16.858	11147.52	0.000	1	0.999	0.000	0.000	-	0.000
Aabd	567 (6025)	12 (555)	0.584	0.317	3.391	1	0.066	1.794	0.963	3.341	0.004
ACEI	2924 (6025)	44 (2880)	0.351	0.232	2.293	1	0.130	1.420	0.902	2.237	0.003
ARB	745 (6025)	10 (735)	0.057	0.341	0.028	1	0.868	1.059	0.542	2.066	0.000
Nitrate	1420 (6025)	41 (1379)	1.328	0.230	33.204	1	<0.001	3.773	2.402	5.928	0.041

Baseline categorical variable	***N*** ***(categorical variable of interest [patients with variable data])***	***N*** ***(categorical variable loop diuretic +ve [categorical variable loop diuretic -ve)***	***B***	***SE***	***Wald***	***df***	***p***	***OR*** ***[Exp (B)]***	***Lower 95% CI for Exp (B)***	***Upper 95% CI for Exp (B)***	***NR^2***
Otherantiang	171 (6025)	5 (166)	0.883	0.469	3.545	1	0.060	2.419	0.964	6.066	0.004
Macrovascular disease	1199 (6025)	41 (1158)	1.550	0.231	45.119	1	<0.001	4.711	2.997	7.405	0.055
LVM >228g	5 (392)	2 (3)	- 0.462	0.919	0.253	1	0.615	0.630	0.104	3.811	0.005

aabd, alpha adrenoceptor drugs; ACEI, angiotensin converting enzyme inhibitors; anbd, adrenergic neurone blocking drugs; ARB, angiotensin II receptor antagonists; caantiht, centrally acting antihypertensive drugs; creat, serum creatinine; Dihydropyridine ccb, dihydropyridine calcium channel blockers; Ks, potassium sparing diuretic therapy; LVM, left ventricular mass; MFSU, metformin-sulphonylurea combination therapy; NSAID, non-steroidal anti-inflammatory drug; otherantiang, other antianginal drugs; peripheral vasodilators, peripheral vasodilator therapy; reninh, renin inhibitors; TZD, thiazolidinedione; TZD + insulin, thiazolidinedione-insulin combination therapy; vasodilat, vasodilator antihypertensive drugs

year after inclusion into their respective cohort. Hence, the overall proportion of patients developing incident HF amounts to 0.0129455 (or 1.29%).

As advised by Peduzzi et al. [609], given the proportion of patients developing incident HF within one year after index thiazolidinedione prescription, the maximum number of covariates that can be included in any model amounted to eight.

(i) Incident heart failure logistic regression model 1

Based on univariate analysis (tables 3.52 and 3.53), and taking into account the number of patients for whom data for each covariate were available, the following predictors (covariates) were included:

- Age (years)
- diabetes duration (years) *(square root transformed data)*
- baseline ALT (IU/L) *(log_e transformed data)*
- baseline albumin (g/L) *(log_e transformed data)*
- baseline serum creatinine > 130 μmol/L
- female gender
- baseline macrovascular disease (composite of coronary artery disease, peripheral artery disease and cerebrovascular disease)
- index thiazolidinedione prescription (vs MFSU)

4690 patients were included into the logistic regression model. Employing a significance level of 0.05, the Wald criterion demonstrated that age, baseline serum albumin, baseline serum creatinine and baseline macrovascular disease made a

significant contribution to prediction (see table 3.54). A test of the full model versus a model with intercept only was statistically significant (chi square 70.293, $p < 0.001$ with df = 4). Hosmer and Leneshow test statistic indicated that the model's estimates fit the data at an acceptable level (chi square 5.661, df = 8, $p = 0.685$). Nagelkerke's $R^2 = 0.113$, effectively indicating a relationship of 11.3% between predictors (covariates) and the prediction (i.e. incident HF within one year of inclusion into the cohort). Prediction success overall was 98.8%.

As shown in the final model (table 3.54), index thiazolidinedione prescription *per se* does not emerge as a significant predictor, suggesting that the risk factors for developing of incident HF are similar to those for patients prescribed metformin-sulphonylurea combination therapy. Once again, Wald's statistics for baseline macrovascular disease suggest it is the strongest predictor. It is associated with more than four times higher risk of progression to incident HF within one year of index metformin-sulphonylurea or thiazolidinedione prescription, assuming all other covariates are unchanged during the observation period. Likewise, a baseline serum creatinine exceeding 130 µmol/L at index metformin-sulphonylurea or thiazolidinedione prescription is the second most strong predictor in this model, being associated with more than two fold higher risk of progression to HF. Each passing year of life is associated with a 4.9 % increased risk of developing incident HF in this scenario. Inverting odds ratios, and holding all other covariates constant, each 1 g/dL reduction in baseline $\log_e$ serum albumin at index metformin-sulphonylurea combination or thiazolidinedione prescription results in an 3.42 fold increased risk of developing incident HF within one year.

Table 3.54 - Incident heart failure binary logistic regression model 1 - final model covariates predicting incident heart failure within one year of exposure to index metformin-sulphonylurea combination or thiazolidinedione therapy*

Final model covariates	*B*	*SE*	*Wald*	*df*	*p*	*Odds ratio [exp (B)]*	*95% CI lower*	*95% CI upper*
Baseline macrovascular disease	1.415	0.268	27.859	1	< 0.001	4.118	2.435	6.966
Age (years)	0.047	0.014	11.304	1	< 0.001	1.049	1.020	1.078
Baseline serum creatinine > 130μmol/L	0.821	0.365	5.074	1	0.024	2.273	1.113	4.644
Baseline serum albumin (g/L)[a]	-1.232	0.552	4.982	1	0.026	0.292	0.099	0.861
Constant	- 5.832	1.648	12.521	1	< 0.001	0.003		

** Baseline covariates included in the model were age, diabetes duration[b], female gender, alanine aminotransferase[a], serum albumin[a], serum creatinine > 130μmol/L, macrovascular disease and index thiazolidinedione therapy (vs metformin-sulphonylurea combination therapy); [a] log_e transformed data; [b] square root transformed data*

Model chi square 70.293, p < 0.001 with df = 4; NR^2 = 0.116; H-L statistic chi square = 5.661, p = 0.685 with df =8; - 2 LL = 587.545; prediction success overall = 98.8%; ROC (AUC) = 0.800 (95% CI 0.754, 0.846), p < 0.001

ROC curve analysis was used to discriminate between positive and negative cases. Concardance index (c-statistic/AUC) for this model amounted to 0.800 (95% CI 0.754, 0.846) (p < 0.001), suggesting that the final model has an ability to distinguish between the two outcome groups.

(ii) Incident heart failure logistic regression model 2

Given the constraints of including additional covariates into the model (discussed above), an additional binary logistic regression model was run to explore the potential impact of baseline haematocrit on the model. Replacing female gender and retaining all other baseline covariates, age, serum albumin, serum creatinine > 130

µmol/L and macrovascular disease remained significant predictors of incident HF within one year in the final model (table 3.55).

Table 3.55 - Incident heart failure binary logistic regression model 2 - final model covariates predicting incident heart failure within one year of exposure to index metformin-sulphonylurea combination or thiazolidinedione therapy*

Final model covariates	*B*	*SE*	*Wald*	*df*	*p*	*Odds ratio [exp (B)]*	*95% CI lower*	*95% CI upper*
Baseline macrovascular disease	1.729	0.332	21.173	1	< 0.001	5.636	2.942	10.797
Age (years)	0.047	0.017	7.835	1	0.005	1.048	1.014	1.082
Baseline serum creatinine > 130µmol/L	1.032	0.395	6.817	1	0.009	2.805	1.293	6.085
Baseline serum albumin (g/L) [a]	-1.337	0.647	4.273	1	0.039	0.263	0.074	0.933
Constant	- 6.021	1.943	9.606	1	0.002	0.002		

**Baseline covariates included in the model were age, diabetes duration b, haematocrit, alanine aminotransferase a, serum albumin a, serum creatinine > 130 µmol/L, macrovascular disease and index thiazolidinedione therapy (vs metformin-sulphonylurea combination therapy); [a] log_e transformed data; [b] square root transformed data*

Model chi square = 67.106, p < 0.001 with df = 4; NR^2 = 0.144; H-L statistic chi square = 6.621, p = 0.578 with df =8; - 2LL = 423.324; prediction success overall = 99.0%; AUC = 0.798 (95% CI 0.752, 0.844), p < 0.001

3.15 Cox regression model: predicting risk factors for incident heart failure events occurring within one year after index metformin-sulphonylurea combination or thiazolidinedione therapy

3.15.1 Univariate Cox regression

On univariate analysis, the following clinical and pathological factors were associated with time to incident HF, as outlined fully in tables 3.56 and 3.57:

Demographics

- age in years [HR 1.066 (95% CI 1.041, 1.090); p < 0.001]
- diabetes duration in years *(square root transformed data)* [HR 1.513 (95% CI 1.219, 1.876); p < 0.001]
- female gender (p = 0.082)

Past medical history

- baseline macrovascular disease (p < 0.001)

Drug history

- baseline potassium channel blocker/aldosterone antagonist therapy (p < 0.001)
- baseline verapamil therapy (p = 0.001)
- baseline diltiazem therapy (p = 0.007)
- baseline beta blocker therapy (p = 0.001)
- baseline alpha adrenoceptor blocking drugs (p = 0.048)
- baseline nitrates (p < 0.001)
- baseline other antianginal drugs (p = 0.051)

Laboratory-based clinical investigations

- baseline haematocrit expressed as % value [HR 0.900 (95% CI 0.845, 0.959); p = 0.001]
- baseline HDL-C concentration in mmol/L *(log_e transformed data)* [HR 3.515 (95% CI 1.203, 10.268); p = 0.022]

- baseline estimated glomerular filration rate in mls/min/1.73m^2 *(log_e transformed data)* [HR 0.984 (95% CI 0.975, 0.992); p < 0.001]
- baseline serum creatinine > 130 µmol/L (p < 0.001)
- baseline serum albumin in g/L *(log_e transformed data)* [HR 0.129 (95% CI 0.048, 0.345); p < 0.001]
- baseline alanine aminotransferase in IU/L *(log_e transformed data)* [HR 0.413 (95% CI 0.245, 0.696); p = 0.001]

3.15.2 Multivariate Cox regression

Based on the outcomes of univariate analysis, Cox regression was used to assess the strength of association between time to incident HF and clinical and pathological risk factors. As outlined in multivariate logistic regression analysis, the maximum number of covariates that could be included in Cox regression analysis, based on the available data, amounted to eight.

(i) Incident heart failure Cox regression model

Variables (covariates) included in multivariate Cox regression analysis were those deemed significant (p < 0.1) on univariate screening (as summarised in tables 3.56 and 3.57), namely:

- Age (years)
- Diabetes duration (years) *(square root transformed data)*
- Baseline haematocrit (%)
- serum creatinine > 130 µmol

Table 3.56 - Univariate Cox regression: baseline continuous independent variable predicting incident heart failure events occurring within one year of inclusion into the metformin-sulphonylurea or thiazolidinedione cohort.

Baseline continuous variable	*N [HF+ (patients with variable data)]*	*B*	*SE*	*Wald*	*df*	*p*	*Hazard ratio [Exp (B)]*	*Lower 95% CI for Exp (B)*	*Upper 95% CI for Exp (B)*	*- 2 Log Likelihood*
Age	77 (6025)	0.063	0.012	29.177	1	<0.001	1.066	1.041	1.090	1291.793
Diabetes duration (years) [a]	77 (6025)	0.414	0.110	14.173	1	<0.001	1.513	1.219	1.876	1308.752
MAP (mmHg)	66 (5302)	- 0.006	0.014	0.166	1	0.684	0.994	0.968	1.022	1116.980
SBP (mmHg)	66 (5302)	0.009	0.008	1.235	1	0.266	1.009	0.993	1.025	1115.937
DBP (mmHg)	66 (5302)	- 0.024	0.015	2.677	1	0.102	0.976	0.948	1.005	1114.471
Weight (kg)	66 (5520)	0.004	0.007	0.272	1	0.602	1.004	0.990	1.017	1122.252
BMI (kg/m^2)	66 (5520)	0.007	0.022	0.094	1	0.759	1.007	0.964	1.052	1122.428
Haematocrit (%)	46 (4525)	- 0.105	0.032	10.747	1	0.001	0.900	0.845	0.959	754.198
HbA1c (%)	68 (5638)	0.120	0.077	2.397	1	0.122	1.127	0.969	1.312	1157.427
Total cholesterol (mmol/L) [b]	66 (5466)	0.748	0.537	1.940	1	0.164	2.114	0.737	6.060	1188.260
HDL-C (mmol/L) [b]	50 (4931)	1.257	0.547	5.283	1	0.022	3.515	1.203	10.268	832.495
LDL-C (mmol/L) [a]	41 (3717)	0.333	0.487	0.467	1	0.494	1.395	0.537	3.627	661.400
Triglycerides (mmol/L) [b]	51 (4267)	- 0.415	0.259	2.556	1	0.110	0.661	0.397	1.098	835.814
ALT (IU/L) [b]	63 (5026)	- 0.884	0.266	11.046	1	0.001	0.413	0.245	0.696	1046.630
Sodium (mmol/L)	69 (5571)	- 0.020	0.042	0.230	1	0.631	0.980	0.902	1.065	1173.750
Egfr ($mls/min/1.73m^2$) [b]	61 (5012)	- 0.016	0.004	13.388	1	<0.001	0.984	0.975	0.992	1009.952
TSH (mIU/L)	57 (4806)	0.009	0.088	0.010	1	0.921	1.009	0.848	1.200	953.295
Serum albumin (g/L) [b]	68 (5278)	- 2.051	0.504	16.597	1	<0.001	0.129	0.048	0.345	1133.048
TZD dose (% maximal) [b]	28 (2549)	- 0.179	0.554	0.104	1	0.747	0.836	0.282	2.478	431.999

Baseline continuous variable	***N*** ***(index loop diuretics prescribed [patients with variable data])***	***B***	***SE***	***Wald***	***df***	***p***	***Hazard ratio*** ***[Exp (B)]***	***Lower 95% CI for Exp (B)***	***Upper 95% CI for Exp (B)***	***- 2 Log Likelehood***
Baseline IVS (cm)	6 (447)	0.682	1.439	0.225	1	0.636	1.978	0.118	33.217	71.841
Baseline LVPW (cm) [b]	5 (397)	0.512	1.976	0.067	1	0.796	1.668	0.035	80.266	58.636

ALT, alanine aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated haemoglobin; Hct, haematocrit; HDL-C, high density lipoprotein cholesterol; IVS, interventricular septum width; LDL-C, low density lipoprotein cholesterol; LVM, left ventricular mass; LVPW, left ventricular posterior wall thickness; MAP, mean arterial pressure; SBP, systolic blood pressure; TSH, thyroid stimulating hormone; TZD thiazolidinedione; [a] square root transformed data; [b] log_e transformed data

Table 3.57 - Univariate Cox regression analysis (Kaplan-Meier survival): baseline categorical independent variables predicting incident heart failure events occurring within one year of inclusion into the metformin-sulphonylurea or thiazolidinedione cohort

			Categorical variable of interest				*Comparator categorical variable*				*Log rank test*		
Baseline categorical variable of interest	***N*** ***(categorical variable of interest [patients with variable data])***	***N*** ***HF +ve (patients with comparator variable data)***	***Mean Survival time***	***SE Survival time***	***Lower 95% CI for survival time***	***Upper 95% CI for survival time***	***Mean Survival time***	***SE Survival time***	***Lower 95% CI for survival time***	***Upper 95% CI for survival time***	***Chi Square***	***df***	***p***
Male gender	3372 (6025)	51 (3321)	362.488	0.407	361.691	363.285	363.273	0.389	362.511	364.036	3.026	1	0.082
Female gender	2653 (6025)	26 (2627)	363.273	0.389	362.511	364.036	362.488	0.407	361.691	363.285	3.026	1	0.082
TZD (vs MFSU)	92 (2549)	2 (90)	359.462	3.876	351.864	367.060	363.413	0.374	362.679	364.147	1.781	1	0.182
TZD + insulin	2549 (6025)	28 (2521)	363.279	0.385	362.525	364.034	362.504	0.405	361.709	363.299	1.089	1	0.297
Creat > 130 µmol/L	68 (5278)	10 (58)	356.268	2.904	350.576	361.960	363.108	0.289	362.541	363.675	17.428	1	< 0.001
Peripheral vasodilators	287 (6025)	2 (285)	363.900	0.811	362.310	365.489	362.777	0.297	362.196	363.358	0.782	1	0.377
Thiazide diuretics	2045 (6025)	24 (2021)	362.948	0.473	362.021	363.876	362.771	0.357	362.073	363.470	0.197	1	0.666
Potassium sp. diuretics	238 (6025)	10 (228)	357.899	2.673	352.660	363.139	363.031	0.276	362.491	363.572	17.448	1	< 0.001
NSAIDs	4224 (6025)	52 (4172)	362.909	0.338	362.246	363.572	362.653	0.529	361.617	363.688	0.178	1	0.673
Dihydropyridine CCBs	2257 (6025)	34 (2223)	362.530	0.493	361.562	363.497	363.011	0.347	362.331	363.691	1.610	1	0.204
Verapamil	96 (6025)	5 (91)	362.115	1.532	359.112	365.118	362.843	0.289	362.278	363.409	11.885	1	0.001
Diltiazem	428 (6025)	13 (496)	359.953	1.505	357.002	362.903	363.095	0.279	362.549	363.641	7.400	1	0.007
Beta blockers	2532 (6025)	46 (2486)	361.719	0.546	360.648	362.790	363.638	0.290	362.069	364.208	10.210	1	0.001
Vasodilat	36 (6025)	1 (35)	356.278	8.600	339.421	373.134	362.870	0.282	362.317	363.423	0.660	1	0.417
Caanitht	94 (6025)	1 (93)	362.725	2.261	358.294	367.156	362.831	0.288	362.267	363.395	0.025	1	0.873
Anbd	13 (6025)	0 (13)	-	-	-	-	-	-	-	-	0.177	1	0.674
Aabd	567 (6025)	12 (555)	361.850	1.053	359.786	363.913	362.928	0.295	362.349	363.507	3.918	1	0.048
ACEI	2924 (6025)	44 (2880)	362.581	0.429	361.740	363.422	363.065	0.378	362.324	363.806	2.546	1	0.111

			Categorical variable of interest				*Comparator categorical variable*				*Log rank test*		
Baseline categorical variable of interest	***N*** ***(categorical variable of interest [patients with variable data])***	***N*** ***HF +ve [patients with comparator variable data])***	***Mean Survival time***	***SE Survival time***	***Lower 95% CI for survival time***	***Upper 95% CI for survival time***	***Mean Survival time***	***SE Survival time***	***Lower 95% CI for survival time***	***Upper 95% CI for survival time***	***Chi Square***	***df***	***p***
ARB	745 (6025)	10 (735)	362.339	0.902	360.570	364.107	362.898	0.300	362.311	363.486	0.067	1	0.796
Nitrates	1420 (6025)	41 (1379)	359.537	0.942	357.691	361.383	363.844	0.233	363.388	364.300	39.136	1	<0.001
Otherantiang	171 (6025)	5 (166)	359.774	2.782	354.320	365.227	362.921	0.282	362.368	363.473	3.794	1	0.051
Macrovascular disease	1199 (6025)	41 (1158)	358.644	1.105	356.479	360.809	363.870	0.224	363.430	364.309	55.665	1	<0.001
LVM > 228g	5 (392)	2 (3)	363.198	1.413	360.429	365.966	361.786	2.052	357.765	365.807	0.230	1	0.631

aabd, alpha adrenoceptor drugs; ACEI, angiotensin converting enzyme inhibitors; anbd, adrenergic neurone blocking drugs; ARB, angiotensin II receptor antagonists; caantiht, centrally acting antihypertensive drugs; dihydropyridine CCB, dihydropyridine calcium channel blockers; potassium sp. diuretics, potassium sparing diuretic therapy; LVM, left ventricular mass; NSAID, non-steroidal anti-inflammatory drug; otherantiang, other antianginal drugs; TZD + insulin, thiazolidinedione-insulin combination therapy; vasodilat, vasodilator antihypertensive drugs

- Baseline serum albumin (g/L) *(log_e transformed data)*
- Baseline alanine aminotransferase (IU/L) *(log_e transformed data)*
- Baseline macrovascular disease
- Index thiazolidinedione prescription (vs metformin-sulphonylurea)

Given baseline serum creatinine > 130 µmol/L, macrovascular disease and index thiazolidinedione prescription defied the Proportional Hazards Assumption, time-dependent variables were constructed for each variable by adding an interaction term that involved $\log_e$ time (days) to index loop diuretic prescription into the Cox model, and testing for its significance. Time-dependent variables were also constructed in the same fashion for age, diabetes duration *(square root transformed data)*, baseline haematocrit, serum albumin *(log_e transformed data)* and alanine aminotransferase *(log_e transformed data)* as evidence that hazard ratios for these covariates do not change over time.

There were no significant interactions between any of the included covariates in this model. Out of a total of 4260 patients, for whom data were available for this model, 44 patients developed incident HF within one year of prescription of metformin-sulphonylurea combination or thiazolidinedione therapy. 4216 patients were censored within the aforementioned period of observation. The covariates as a set reliably improved the predictability of the Cox regression model (chi square 2111.312, $p < 0.001$ with df = 13). The standard error (SE) of each variable included in the model was small, suggesting no significant multicollinearity. The Wald criterion demonstrated that (in decreasing order of importance) age, baseline haematocrit, serum albumin, baseline macrovascular disease, and baseline alanine

aminotransferase and their respective interactions with time made a significant contribution to predicting time to index loop diuretic prescription in this setting, as outlined in table 3.58. Baseline serum creatinine was a marginally significant covariate ($p = 0.05$). Covariate*time interactions suggested a decreasing hazard ratio over time for baseline macrovascular disease, alanine aminotransferase, serum creatinine and serum albumin. Hazard ratios for age and haematocrit, remained stable over the period of observation, in keeping with log-minus-log plots which had suggested that each of the latter covaariates satisfied the Proportiona Hazards Assumption (table 3.59, figure 3.15)

There were insufficient data to permit modelling baseline drug therapy, left ventricular mass or interventricular septum width as covariates in a Cox regression model predicting incident HF events.

Table 3.58 - Incident heart failure Cox regression model 1 predicting incident heart failure events within one year of inclusion into the metformin-sulphonylurea combination or thiazolidinedione cohort.

Final baseline model covariates	*B*	*SE*	*Wald*	*df*	*p*	*Hazard ratio*	*95% CI lower*	*95% CI upper*
Age (years)	1.777	0.271	42.850	1	<0.001	5.911	3.472	10.062
Haematocrit (%)	2.310	0.560	17.015	1	<0.001	10.070	3.361	30.174
Serum creatinine > 130 µmol/L	11.015	5.610	3.855	1	0.050	60750.163	1.020	3619757003
Serum albumin (g/L) [a]	35.320	8.811	16.068	1	<0.001	2.184E+15	69049468.21	6.907E+22
ALT (IU/L) [a]	17.043	5.614	9.217	1	0.002	25217422.95	420.051	1.514E+12
Macrovascular disease	15.773	5.071	9.674	1	0.002	7079269.741	341.613	1.467E+11
Age(years)*$\log_e$time	-0.317	0.049	41.449	1	<0.001	0.728	0.661	0.802
Haematocrit (%)*$\log_e$time	-0.422	0.102	17.091	1	<0.001	0.656	0.537	0.801
Serum creatinine > 130 µmol/L*$\log_e$time	-1.939	1.064	3.323	1	0.068	0.144	0.018	1.157
Serum albumin (g/L)*$\log_e$time	-6.689	1.656	16.306	1	<0.001	0.001	<0.001	0.032
ALT(IU/L) *$\log_e$time	-3.058	1.033	8.762	1	0.003	0.047	0.006	0.356
Macrovascular disease*$\log_e$time	-2.532	0.929	7.422	1	0.006	0.080	0.013	0.492
TZD (vs MFSU)*$\log_e$time	0.172	0.071	5.876	1	0.015	1.187	1.033	1.364

**Baseline covariates included in the model were age, diabetes duration [b], haematocrit, serum creatinine > 130 µmol/L, serum albumin [a]; alanine aminotransferase [a], macrovascular disease, index thiazolidinedione (vs metformin-sulphonylurea therapy)*
[a] loge transformed data; [b] square root transformed data

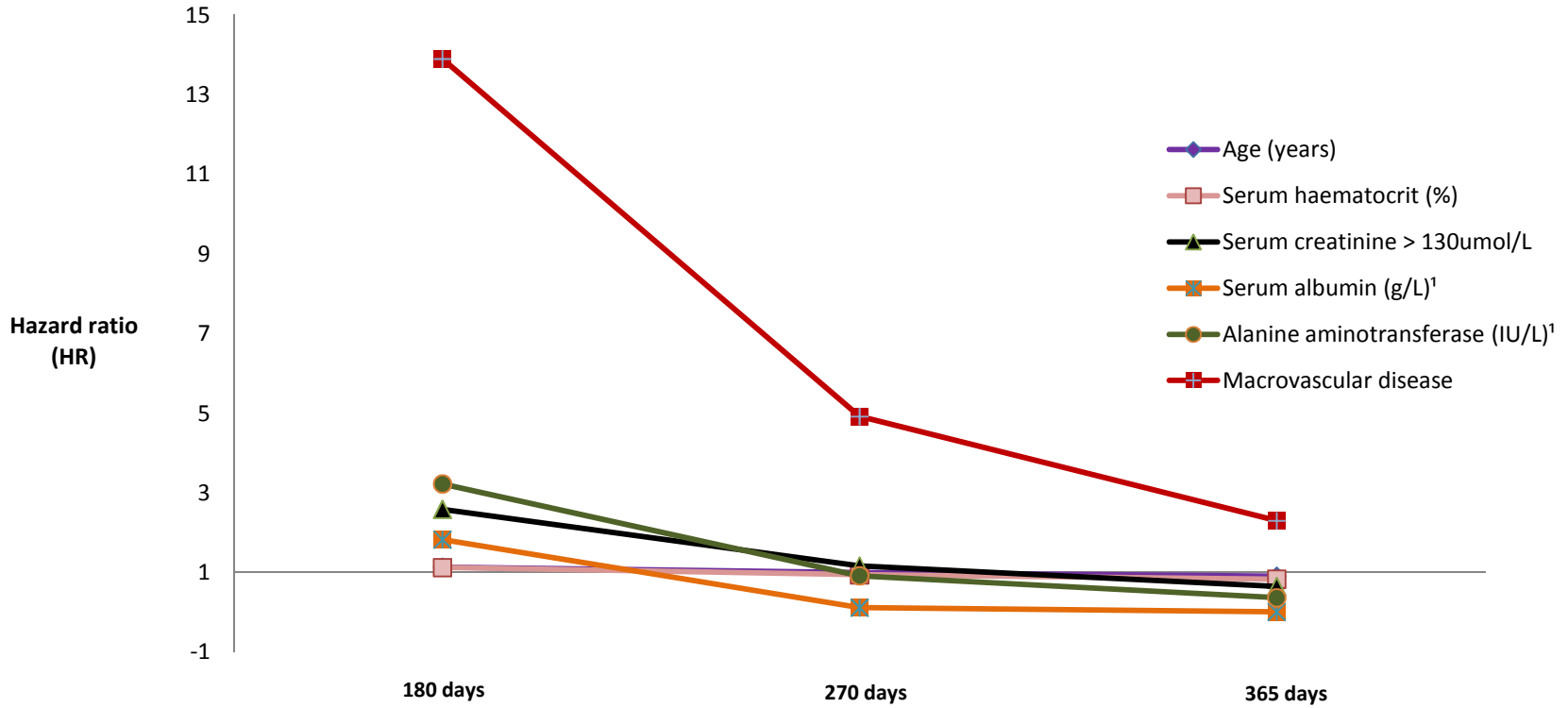
Events = 44 censored = 4216; - 2 LL = 230.664; Model chi square = 2111.312, p < 0.001 with df = 13

Table 3.59 - Incident heart failure Cox regression model 1 predicting incident heart failure within one year of inclusion into the metformin-sulphonylurea or thiazolidinedione cohort. Variation of estimated hazard ratios (HR) is given at three monthly intervals (six months, nine months and one year) for all covariates.

Time-dependent covariates	*HR at 6 months (180 days)*	*HR at 9 months (270 days)*	*HR at 12 months (365 days)*
Age (years)	1.141	1.002	0.911
Haematocrit (%)	1.127	0.948	0.835
Serum creatinine > 130 µmol/L	2.590	1.170	0.654
Serum albumin (g/L)[a]	1.830	0.118	0.016
Alanine aminotransferase (IU/L)[a]	3.228	0.921	0.368
Macrovascular disease	13.900	4.922	2.303

[a] *$\log_e$ transformed data*

Figure 3.15 - Variation in hazard ratio values for incident heart failure developing within one year of index metformin-sulphonylurea combination or thiazolidinedione prescription. Data are plotted at 180, 270 and 365 days. Patients treated with metformin-sulphonylurea combination or thiazolidinedione therapy for less than 90 days were excluded.



[1] Log_e transformed data

3.16 Do CYP2C8*3 and *4 genotypes infer a reduced oedematogenic risk following thiazolidinedione exposure?

Out of a total of 2664 thiazolidinedione-treated patients, CYP2C8 data were available for 1309 patients. Of these, 318 (24.3%) carried the CYP2C8*3 allele (whether homozygotes, heterozygotes or compound heterozygotes with CYP2C8*4), while 120 (9.2%) carried the CYP2C8*4 allele. Expressed differently, 888 (76.8%) were wild type carriers (CYP2C8 *1/*1), 372 (28.4%) were heterozygotes for the *3 or *4 allele (CYP2C8 *1/*3 or *1/*4), whereas 49 (1.8%) were homozygotes or compound heterozygotes (CYP2C8 *3/*3, *3/*4 or *4/*4)

There were no significant differences in the frequencies of index loop diuretic prescription or incident HF rates (occurring within one year of index thiazolidinedione prescription) between patients carrying at least one copy of the CYP2C8*3 or CYP2C8*4 allele and wild type carriers (CYP2C88 *1/*1) (Fisher exact test p = 0.483, 0.185 respectively). Likewise, as outlined in tables 3.60 and 3.61 below, the frequency of occurrence of index loop diuretic prescription and incident HF was similar across heterozygous (CYP2C8 *1/*3 or CYP2C8 *1/*4), compound heterozygous (CYP2C8 *3/*4) and homozygous (CYP2C8 *3/*3 or CYP2C8 *4/*4) subgroups (compared to wild type carriers).

Univariate logistic regression did not identify CYP2C8*3 or *4 variants as being significant risk factors for the outcomes of interest, whether in the heterozygous or homozygous state (tables 3.62 and 3.63 below).

Table 3.60 - Number (%) of patients treated with an index loop diuretic within one year after inclusion into the thiazolidinedone cohort.

	CYP2C8 genotype variant		
	****1/*1***	****1/*3 or *1/*4***	****3/*3 or *3/*4 or *4/*4***
Index loop - ve	658 (95.8)	290 (96.7)	33 (94.3)
Index loop +ve	29 (4.2)	10 (3.3)	2 (5.7)
Loop data missing	201	72	14
Total	888	372	49

Chi Square = 0.700, p = 0.705 with df = 2

Table 3.61 - Number (%) of patients developing heart failure within one year after inclusion into the thiazolidinedone cohort.

	CYP2C8 genotype variant		
	****1/*1***	****1/*3 or *1/*4***	****3/*3 or *3/*4 or *4/*4***
HF - ve	847 (98.8)	350 (98.6)	46 (100)
HF +ve	10 (1.2)	5 (1.4)	0 (0)
HF data missing	31	17	3
Total	888	372	49

Chi Square = 0.701, p = 0.705 with df = 2

Table 3.62 - Univariate binary logistic regression predicting index loop diuretic prescription within one year of index thiazolidinedione therapy.

Baseline categorical variable	*N (index loop diuretics prescribed [patients with variable data])*	*B*	*SE*	*Wald*	*df*	*p*	*Odds ratio (Exp [B])])*	*Lower 95% CI for Exp (B)*	*Upper 95% CI for Exp (B)*
CYP2C8 *3 variant	9/248	- 0.136	0.385	0.124	1	0.724	0.873	0.411	1.855
CYP2C8 *4 variant	3/301	- 0.341	0.609	0.313	1	0.576	0.711	0.216	2.347
CYP2C8 *3 or CYP2C8 *4 variant	12/335	- 0.171	0.350	0.238	1	0.625	0.843	0.425	1.674
CYP2C8 *3/*3 (vs no *3)	1/16	0.436	1.048	0.173	1	0.678	1.546	0.198	12.067
CYP2C8 *4/*4 (vs no *4)	1/5	1.759	1.130	2.423	1	0.120	5.809	0.634	53.233

Table 3.63 - Univariate binary logistic regression predicting incident heart failure within one year of index thiazolidinedione therapy.

Baseline categorical variable	*N (incident heart failure [patients with variable data])*	*B*	*SE*	*Wald*	*df*	*p*	*Odds ratio (Exp [B])])*	*Lower 95% CI for Exp (B)*	*Upper 95% CI for Exp (B)*
CYP2C8 *3 variant	4/302	0.142	0.588	0.059	1	0.808	1.153	0.364	3.648
CYP2C8 *4 variant	1/115	- 0.346	1.040	0.111	1	0.739	0.707	0.092	5.429
CYP2C8 *3 or CYP2C8 *4 variant	5/401	0.067	0.551	0.015	1	0.903	1.069	0.363	3.150
CYP2C8 *3/*3 (vs no *3)	0/23	- 16.750	8380.814	0.000	1	0.998	0.000	0.000	-
CYP2C8 *4/*4 (vs no *4)	0/23	- 18.813	15191.515	0.000	1	0.999	0.000	0.000	-

3.17 Discussion

This study has identified risk factors for index loop diuretic prescription and incident HF in a cohort of T2DM patients treated with the most commonly prescribed anithyperglycaemic combination therapy (metformin and sulphonylureas) and thiazolidinediones. Importantly, available data suggest that risk factors for index loop diuretic prescription (a surrogate marker of fluid retention) and incident HF are shared between patients in both treatment categories, and that neither index metformin-sulphonylurea combination therapy nor thiazolidendione prescription are risk factors for these adverse events on multivariate analysis.

To my knowledge, this is the first study comparing incident HF rates in these two treatment subgroups, as most available data have compared thiazolidinediones solely with monotherapy / placebo comparators. RECORD, an open-label prospective trial randomising T2DM patients inadequately controlled on metformin or sulphonylurea monotherapy to add-on rosiglitazone or metformin-sulphonylurea combination therapy, is a notable exception. The approach in the present study may be more generalisable as it mirrors clinical practice, particularly given the reported differential effects of metformin and sulphonylureas on incident HF events, and recurrent HF in T2DM patients with established HF.

Given the unanticipated difficulties recruiting patients for my clinical study, analysis of population-based data of clinically significant peripheral oedema necessitating index loop diuretic therapy was a novel approach to unravelling the mechanisms underpinning thiazolidinedione-associated fluid overload. Based on available

evidence, one may consider this surrogate marker of fluid overload as a sentinel sign of clinical HF or (unexplained) peripheral oedema [610]. In PROactive, 27.4% of patients randomised to pioglitazone reported oedema [vs 15.9% (placebo); < 0.001), while 21.6% developed serious or nonserious oedema without HF [vs 13.0% (placebo); p < 0.001]. Oedema preceded HF in 34.2% and 24.1% of patients randomised to pioglitazone and placebo respectively [261]. An insulin comparator subgroup was included as a valuable source of descriptive data, given the reported association of insulin therapy with fluid overload [352, 611-613], but was not included in multivariate analysis, given that insulin therapy is generally reserved for patients at a more advanced stage of T2DM.

The relative frequency of prescription of thiazolidinediones to patients with established HF (4.32%), albeit lower than for metformin-sulphonylurea combination (6.21%) and insulin therapy (17.60%) is rather surprising, given the unequivocal advice voiced by multiple clinical practice guidelines. Nonetheless, thiazolidinedione prescription among such patients was lower than that reported among Medicare beneficiaries (7.1% for patients prescribed between 1998-1999 and 16.1% for those prescribed between 2000-2001) [614]. Similarly, a retrospective analysis of 24 746 elderly Korean patients with T2DM reported that thiazolidinediones were prescribed to 10.4% of patients with established HF and 8.8% of patients without [615].

While following similar trends, thiazolidinedione prescription rates among patients prone to oedema (and hence loop diuretic prescription) were even higher (21.28%). Differences generally reached statistical signifance with either comparator cohorts on *post-hoc* testing. There were no differences in background use of loop diuretics

between metformin-sulphonylurea and thiazolidinedione-treated female patients, and for background HF rates between metformin-sulphonylurea and thiazolidinedione-treated male patients. Results of the latter two *post-hoc* analyses may have been limited by sample size, and do not necessarily reflect gender related differences in prescription practices. Despite the reported association between insulin therapy and fluid retention / HF [352], insulin prescription was necessarily more likely in patients prone to cardiovascular disease and renal impairment, possibly as a consequence of progressive beta-cell exhaustion precipitating inadequate glycaemic control on established oral glucose lowering agents.

This study reported that 1.1% of patients develop incident HF within one year of their thiazolidinedione prescription. This rate was considerably lower than for insulin therapy (3.5%), and comparable to metformin-sulphonylurea combination therapy (1.4%). This study's reported incident HF rates for thiazolidinedione-treated patients (rosiglitazone / pioglitazone) was virtually twice that reported in the DREAM trial (rosigitazone, 0.54%), comparable to HF events in ADOPT (rosligitazone, 1.51%) and considerably lower than those reported in RECORD (rosiglitazone, 2.7%) and PROactive (pioglitazone, 10.7%). 4.3% of patients in this study's cohort required prescription of an index loop diuretic (a surrogate marker of oedema) within one year of exposure to a thiazolidinedione. This is considerably lower than that reported in DREAM (6.6%), ADOPT (14.1%) and ProACTIVE (21.6%). However, as has been ascertained in the introductory chapter, these four prospective trials recruited patients with a spectrum of glycaemia and cardiovascular risk, ranging from prediabetes (DREAM) to pharmacologically naïve T2DM (ADOPT), high risk T2DM inadequately controlled on metformin or sulphonylurea monotherapy

(RECORD) and T2DM treated with diet or oral glucose lowering agents or insulin (PROactive).

Comparisons of incident HF rates need to be made with caution, given differences in HF definitions across these four prospective trials. Moreover, none of these trials were primarily designed to investigate oedema and HF rates. All four prospective trials captured incident HF events for a longer time period ranging from 34.5 months (PROactive) to 66 months (RECORD), and did not report outcomes after one year of treatment. Oedema outcomes were likewise reported at the end of the observation period in all four prospective trials (except RECORD). Given the published effects of rosiglitazone and pioglitazone on other macrovascular outcomes, capturing incident HF and index loop diuretic prescription within one year of prescription of a thiazolidinedione (or comparator drug) was more likely to yield unbiased information on the outcome of interest.

Unlike these four prospective clinical trials, this study's retrospective analyses did not permit a comparison of incident HF events between rosiglitazone and pioglitazone-treated patients. Patients recruited in these prospective trials were generally younger (mean range 54.7 [DREAM] to 61.8 [PROactive] years vs mean [SD] = 63.23 [9.77] years for this study). Additionally, patients recruited in each study were characterised by a relatively homogenous cardiovascular risk (ranging from low-risk pre-diabetes [DREAM] to high risk T2DM patients [PROactive]). As 15.1% of patients were known to suffer from macrovascular disease at index thiazolidinedione prescription, this study's cohort encompasses T2DM patients with a range of cardiovascular risk, akin to that in a T2DM population. This study's

observations of higher (unadjusted) incident HF rates for insulin-treated T2DM are consistent with those reported by several observational studies on multivariate adjustment [239, 277, 616].

Age, BMI, systolic blood pressure, haematocrit, alanine aminotransferase and macrovascular disease emerged as significant baseline predictors of time to oedema requiring loop diuretics on Cox regression analysis. Age, haematocrit, serum creatinine > 130 µmol/L (borderline significance), serum albumin, alanine aminotransferase and macrovascular disease emerged as significant baseline predictors of time to incident HF within one year of inclusion into the metformin-sulphonylurea combination or thiazolidinedione cohort. Modelling incident HF events generally validated this study's predictors for loop diuretic prescription, and is consistent with the observation that diuretic use predicts HF in T2DM patients randomised to pioglitazone or placebo in the PROactive trial [261].

Importantly, thiazolidinedione prescription did not emerge as a significant contributor to fluid retention requiring loop diuretics and HF on univariate or multivariate analysis, suggesting that risk factors for developing these adverse events following index thiazolidinedione prescription are shared with patients prescribed metformin-sulphonylurea combination therapy. These results contrast sharply with those reported in a *post-hoc* analysis of data from RECORD and PROactive for rosiglitazone and pioglitazone respectively [258, 261]. Both ascribed an increased risk for the respective thiazolidinedione on multivariate *post-hoc* analyses. However, RECORD investigators excluded patients awaiting a cardiovascular intervention, those hospitalized for a major cardiovascular event within the previous three months

and individuals with renal and/or liver impairment, uncontrolled hypertension or an HbA1c of <7% / >9% [153]. Likewise, PROactive excluded patients on insulin monotherapy and those with severe peripheral vascular disease, end-stage renal disease requiring haemodialysis, significantly elevated alanine aminotransferase, and subjects awaiting coronary or peripheral arterial revascularisation [256]. This approach will have exluded patients at higher a priori risk of incident HF, introducing selection bias into the *post-hoc* models. Patients recruited into the PROactive trial were randomised to a placebo rather than active comparator. In agreement with this study's findings, neither metformin nor sulphonylureas emerged as significant predictors of incident congestive HF in multivariate Cox regression analysis of T2DM US patients [239]. Toprani et al. were also reassuring in this regard, ascribing a decreased risk of thiazolidinedione associated incident HF [273].

This study's findings are consistent with differences in baseline characteristics between patients progressing to index loop diuretic prescription / incident HF and those who did not. The association between age and incident HF has long been established [617, 618], both in diabetic cohorts [219, 239, 277-279, 619], and in the general population [620-625], and has been replicated in this study. Likewise, baseline macrovascular disease predicted index loop diuretic prescription and incident HF events in this Tayside cohort. This is consistent with results from studies analyzing new-onset HF events in patients whose T2DM was complicated by coronary artery disease [219, 239, 277, 278], peripheral artery disease or stroke [219, 278], and in individuals recruited from population based cohorts who suffered from coronary artery disease at baseline [235, 621, 623, 624, 626-628].

In general, this study's reported findings for age, albumin, systolic blood pressure, serum creatinine and macrovascular disease follow those reported in the Health ABC Heart Failure Score for elderly (diabetic and non-diabetic) patients [629]. Nichols et al. published similar findings in a cohort of 8231 patients with T2DM, additionally attributing an increased incident HF risk to diabetes duration, baseline BMI, mean HbA1c, insulin use, gross proteinuria, end-stage renal disease and mean DBP, and a (surprisingly) lower risk for microalbuminuria [239]. The authors had ascribed the latter finding to a confounding effect of ACE inhibitors. One does not exclude that this may also have masked the effect of baseline serum creatinine on the final model for index loop diuretic prescription in this study, in patients prone to, but not yet developing clinical HF, given the strong association on univariate analysis ($p < 0.001$). Given recommendations that metformin should be used with caution in individuals with moderate renal impairment (eGFR 30-45 mls/min/1.73 m^2), and is contraindicated in patients with severe renal impairment (eGFR < 30 mls/min/1.73 m^2) [630], it is possible that metformin was not prescribed in patients with poor renal function who are particularly prone to lactic acidosis and HF.

There were no significant differences in baseline HbA1c between metformin-sulphonylurea combination and thiazolidinedione cohorts, or between loop diuretic and HF categories. Most patients in either cohort had suboptimal baseline glycaemic control. Additionally, baseline HbA1c did not predict index loop diuretic prescription or incident HF on univariate analysis. The association between glycaemic control and incident HF in T2DM is somewhat complex. While Nichols et al. reported no association with baseline measurements, reduction in HbA1c values averaged over the 30 month follow-up period predicted incident HF, suggesting a

role for cumulative rather than recent glycaemic burden [277]. The data in the present study concurs with this observation, particularly given that outcomes of interest were analysed at a relatively short time after baseline HbA1c measurement. In contrast, analysis of data from the PROactive trial revealed that a baseline HbA1c exceeding 7.4% predicted incident HF during a mean (± SD) follow-up period of 34.5 (± 2.3) months [261]. Several other studies suggest that poorer glycaemic control is associated with higher incidence rates for HF, both in diabetic and in non-diabetic patients [212, 218, 238, 631, 632]. A recently published systematic review and meta-analysis of ten prospective epidemiological studies comprising 178 929 participants and 14 176 incident congestive HF cases ascribed an overall adjusted risk ratio for CHF of 1.15 (95% CI 1.10, 1.21) for each percentage point higher HbA1c. However, there was significant heterogeneity between the studies, not explained by available study-level characteristics [633].

This study's reported lack of association between female gender and oedema on multivariate analysis are consistent with those reported for incident HF on multivariate survival analysis by Nichols et al. [239]. In contrast, Maru and colleagues had reported that type 2 diabetic males were at an increased risk of incident HF within the first year of diagnosis across all age groups [278]. However, this study’s observation period antedated the introduction of thiazolidinediones into the European market, and was restricted to metformin, sulphonylureas, acarbose, guam gum and insulin (monotherapy or in combination). Concomitant use of insulin and thiazolidinediones did not predict oedema requiring loop diuretic treatment or incident HF on univariate analysis in this study. These results need to be interpreted with caution, given that, as expected, only 70 patients had been prescribed

thiazolidinediones in combination with insulin. Nonetheless, *post-hoc* analysis of PROactive data showed that baseline insulin therapy did not predict serious HF events on multivariate analysis, despite observations that serious HF occurred more frequently in patients treated with insulin at baseline, irrespective of pioglitazone or placebo [261].

BMI emerged as a significant predictor of time to index loop diuretic prescription on multivariate analysis. Rather surprisingly, this covariate was not significant on univariate analysis for incident HF events. The latter observation may have been limited by the small number of HF events in this dataset. Nonetheless, this study's observations for index loop diuretic prescription are consistent with those reported for incident HF in the Framingham study [634, 635], NHANES I [235] and in a community-based elderly cohort [636]. In contrast, obesity did not remain a significant predictor of incident HF when correcting for insulin resistance (measured as euglycaemic clamp glucose diposal rate) [626] or inflammatory markers (interleukin-6 or C-reactive protein) [620] in other studies.

As a major determinant of prevalent oncotic pressure, serum albumin would be expected to influence the threshold for pulmonary oedema in response to an elevation in left atrial pressure. Filippatos et al. demonstrated that baseline hypoalbuminaemia (defined as < 3.5 g/dL) predicts incident HF in community dwelling older adults without baseline evidence of this disease entity during ten years of follow-up [637]. Analysis of data from the Health, Aging and Body Composition Study revealed that baseline serum albumin concentrations are inversely related to incident HF events in a time-dependent manner, even when

controlling for inflammatory markers, incident coronary heart disease [638]. In this prospective study (median follow-up 9.4 years), Gopal et al. demonstrated that participants developing incident HF earlier were characterised by a lower serum albumin concentration than individuals developing HF over the remaining observation period [638]. Patients requiring an index loop diuretic within one year of index metformin-sulphonylurea combination or thiazolidinedione therapy were characterised by lower baseline serum albumin concentrations in this study's cohort. This study's time-to-event data consistently confirm this inverse relationship for both incident HF and index loop diuretic prescription at a relatively early stage of oral glucose lowering agent exposure (one year) across all models. Morevoer, this association holds true even for T2DM patients whose baseline serum albumin hovers within the normal range [mean (SD) baseline serum albumin = 43.51 (3.55) g/L (metformin-sulphonylurea combination), 44.00 (2.88) g/L (thiazolidinediones)].

ALT was identified as a predictor of time to index loop diuretic prescription and incident HF events. This relationship exhibited a sigificant time-varying effect which mirrors that seen for macrovascular disease. The increased risk associated with ALT is largely seen in the first six to nine months after index metformin-sulphonylurea combination or thiazolidinedione therapy, and subsequently wears off to become a protective effect. The initial increased risk is consistent with ALT's association with non-alcoholic liver disease [639], endothelial dysfunction [640] and carotid atherosclerosis [641]. Moreover, ALT has been shown to predict coronary artery disease events independently of other risk factors [642, 643], including its association with the metabolic syndrome [644]. This remarkable time-varying effect could be explained by the insulin-sensitizing actions of metformin and

thiazolidinediones. A lower baseline ALT would be consistent with greater insulin sensitivity, and could suggest a greater response to metformin and/or thiazolidinediones, rendering such patients increasingly prone to thiazolidinedinone-associated fluid overload.

Prevalent haematocrit levels have been associated with cardiovascular events in a few studies [645, 646]. A higher haematocrit concentration, even within the normal range, has recently been associated with an increased risk of new-onset HF in an observational study capturing data from 3523 patients aged 50 to 65 years who had been enrolled in the Framingham Heart Study [647]. Coglianese et al. partly ascribed their observations to haemoconcentration-associated endothelial dysfunction. This study's time-to-event data for both index loop diuretic prescription and incident HF seemingly concur with these observations within the first six to nine months of index metformin-sulphonylurea combination or thiazolidinedione therapy.

Logistic and Cox regression models identified baseline nitrates as predictors of index loop diuretic prescription and time to index loop diuretic prescription. These data partially concur with those reported by McAlister et al. in their retrospective study of 5631 newly diagnosed T2DM patients [279], who additionally ascribed an increased risk to baseline beta-blockers and a reduced risk to ACE inhibitors and ARBs. Beta-blockers did not emerge as significant univariate predictors of index loop diuretic prescription ($p = 0.076$) and time to index loop diuretic prescription ($p = 0.066$) in this study's dataset. Additionally they were not significant on multivariate analysis. Baseline ACE inhibitors, ARBs or thiazolidinedione-insulin combination therapy were not significant on univariate analysis. Similarly, *post-hoc* analysis of data from

the PROactive study reported no significant excess incident HF events among patients randomised to treatment with pioglitazone on a background of nitrates, ACE inbibitors /ARBs or insulin [648]. Percentage maximum thiazolidinedione dose was not included in multivariate modelling of index loop diuretic prescription (despite a p value of 0.074 on univariate logistic regression), so as not to restrict the model to thiazolidinedione-treated patients.

In general, the present results are consistent with those reported by Castagno et al. [649]. In a meta-analysis of HF events from the PROactive, ACCORD, VADT and RECORD trials, these authors reported that patients allocated intensive glycaemic control using high dose thiazolidinediones were more likely to develop incident HF compared with those receiving low dose therapy [649]. Such a dose-dependent effect was not seen when analysing for metformin and sulphonylureas; neither was it investigated in this study's dataset.

The present study reported that baseline left ventricular mass (a surrogate measure of left ventricular hypertrophy) [603] predicted index loop diuretic prescription ($p = 0.015$) and time to index loop diuretic prescription ($p = 0.006$) on univariate modelling for metformin-sulphonylurea combination or thiazolidinedione-treated patients (there were insufficient data for multivariate modelling). This is consistent with this study's observation of higher baseline left ventricular mass values for index loop diuretic requiring patients on univariate in both cohorts. While left ventricular hypertrophy has been identified as a risk factor for incident congestive HF at a population level in several studies [620, 621, 650], there are no such associations in T2DM patients. The present study's observations generally agree with data

suggesting that T2DM patients are characterised by a higher mean left ventricular mass (even in the absence of hypertension, albumunuria and apparent ischaemic heart disease) [651, 652], and are thus more likely to have clinically inapparent left ventricular dysfunction [653].

In conclusion, given the paucity of evidence from prospective clinical trials, an epidemiological observational study was undertaken to provide information to clarify the relationship between fluid overload, HF and thiazolidinedione exposure. This study identified clinically relevant and applicable prediction models in a well characterised, typical T2DM population inherently at risk of HF, exposed to treatment with first, second and third line oral glucose lowering agents. Most of the risk factors are potentially modifiable, providing an opportunity at risk assessment, close follow-up of at risk patients and aggressive clinical risk management. Moreover, given that most patients have multiple risk factors in various combinations, multivariate modelling is likely to be more robust in predicting individual risk.

Despite its limitations, the retrospective cohort approach offered a valuable insight into prescribing practices in Tayside, and minimised the possibility of selection bias. Given the widely reported association between thiazolidinediones and HF/oedema, the possibility cannot be excluded that high risk patients were barred from thiazolidinedione exposure by prescribers (negative allocation bias), and that this may have impacted on the results of the present study's multivariate models. Moreover, patients may have discontinued their thiazolidinedione therapy soon after their prescription on account of perceived or real harm. The present study sought to

control for this in its cohort definitions by including only patients whose initial thiazolidinedione prescription was followed by at least another prescription within three months. Patients were additionally exluded from a cohort if they had been treated with the same antihyperglycaemic agent within the previous year. Inclusion of a metformin-sulphonylurea combination therapy control cohort in multivariate analysis allowed contextualisation of any hypothesised thiazolidinedione effect by comparing it to ‘standard’ ‘first’ and ‘second line’ glucose lowering agents. Access to accurate drug dispension records ensured that the cohorts are representative of true drug use in the population being examined while minimising misclassification of exposure. Notwithstanding the limitations imposed by retrospective research analysis, this study's approach permitted good characterisation of reasonably extensive covariate data. Including index thiazolidinedione (vs metformin-sulphonylurea combination) therapy as a covariate mitigated any measured or unmeasured baseline differences between either treatment cohort, and avoided the need for propensity scoring.

Nonetheless, the potential existence of other unrecognised and unmeasured counfounding variables cannot be excluded, particularly given the paucity of reported data predicting susceptibility to thiazolidinedione and metformin-sulphonylurea combination therapy induced fluid retention / HF in the literature. The present study sought to minimise (albeit not eliminate) this risk by including as many significant covariates as possible in multivariate modelling. The relative infrequency of incident HF events in the combined metformin-sulphonylurea and thiazolidinedione cohorts inevitably imposed restrictions on the maximum number of covariates that could be included into any one model. This study did not capture data

on race, cigarette smoking, physical activity, electrocardiography, hypoglycaemia and cardiac valvular dysfunction, all of which have been implicated to influence propensity for HF in other studies [235, 627, 654, 655]. Nonetheless, this study's approach permitted recruitment of a larger sample, analysis of sequence of events surrounding outcomes of interest, and the inclusion of a larger number of potential confounders than would have been possible in a prospective trial.

In conclusion, on the basis of the present population-based data, thiazolidinediones *per se* do not appear to contribute significantly to the risk of HF or index loop diuretic prescription (as a surrogate for oedema). Risk factors for such adverse events occurring after index thiazolidinedione exposure are common to patients exposed to index metformin-sulphonylurea combination therapy. Careful patient selection may mitigate these adverse outcomes.

Chapter 4

Systematic review and meta-analysis

Is there a role for adjunct metformin in type 1 diabetes?

Chapter 4 - Systematic review and meta-analysis

Is there a role for adjunct metformin in type 1 diabetes?

Section I - Methods

4.1 Eligible studies

This objective was to capture all trials of metformin in T1DM which were i) randomised, ii) used a treatment duration of at least one week, iii) used either a comparator drug, placebo or used a crossover design, and iv) included consenting patients. This study extracted any data on cardiovascular disease, HbA1c, body weight or BMI, insulin dose, lipids and adverse effects.

4.2 Search strategy

All publications pertaining to T1DM and metformin for any outcomes were captured as follows in PubMed (1950 to week 4th January 2009, updated 6th October 2009) and EMBASE (1974 onwards). The search was conducted as follows using medical search headings (MeSH):

1. "Diabetes Mellitus, Type 1"[MeSH]
2. (DIABET*) AND (TYPE 1[TW] OR IDDM[TW] OR ("INSULIN DEPENDENT" not "NON-INSULIN DEPENDENT"))
3. 1 OR 2
4. "Metformin"[MeSH]
5. metformin [TW]

6. 4 OR 5

The abstracts of all identified publications were manually searched for studies that attempted to evaluate the effect of metformin on any clinically relevant outcome whether in a randomised trial or open label or other design. The citations of all relevant publications were manually searched for any additional studies. Where uncertainty existed, the full text of the article was obtained and reviewed. All potentially relevant studies were assessed and data extraction performed. The resulting tables of evidence were then reviewed. Disagreement was resolved by discussion with Professor John Petrie and Professor Helen Colhoun; independent adjudication was not required.

In addition all ongoing and unpublished trials were searched as follows:

- Cochrane Library 2009 issue 1
- Science Citation Index meeting abstracts (includes European Association for the Study of Diabetes and American Diabetes Association meetings) 1980-October 2008
- Diabetes UK meeting abstracts 2002-2008 Endocrine Society Abstracts 2005-2008
- Science Citation Index meeting Abstracts 1980-2008
- National Research Register (NRR)
- Controlled Trials.com

On the United Kingdom NRR, five trials were registered, all with glycaemic/ metabolic outcomes with end dates in 2005 or earlier. All were emailed to request data:

N0176113569: Completed but unpublished (pilot study).

N0231133055: Completed and published [656].

N0394131469: Not completed.

N0301111201: Completed and published [657].

N0046091476: Not completed.

An online reference to trial N0394131469, initially accessed in the first search (week 4th January 2009), was no longer accessible on searching across multiple research registers on relevant websites (www.nrr.org.uk; www.controlled-trials.com) in the updated search (6th October 2009).

On the controlled-trials.com meta-register, one additional glycaemic/ metabolic trial was found:

NCT00145379: Not completed, still recruiting (n=50).

4.2.1 Subjects

Participants were those of any age described by the authors of the publications as having T1DM or insulin dependent diabetes or youth onset diabetes.

4.2.2 Analysis

A decision was made to summarise the data mostly in text and tabular form since there was obvious heterogeneity between studies in methods, design and outcome measures. However, some data were also presented using standard meta-analysis techniques [658]; the two trials of very short duration [659, 660] were excluded from these. Strictly speaking these formal meta-analysis techniques should only be used when a group of studies is sufficiently homogeneous in terms of participants, interventions and outcomes to provide a meaningful summary [658]. Nevertheless, it was considered useful to have a measure of the statistical significance of apparent effects.

With these *caveats*, a fixed effects model using the inverse variance method was fitted to give a crude measure of the overall treatment effect, to assess its statistical significance and to assess the heterogeneity of treatment effect between studies. Outcomes of effect on %HbA1c and on insulin dose were also examined. The metan STATA user command was used, which quantifies heterogeneity using the I-squared measure [661]. Of the eight eligible studies, one study [657] was excluded as it may have been incorrectly analysed as if it were a parallel group study (in which case the standard deviations will not be valid). Three other studies could not be included as they either did not report the outcomes of interest [659, 660], or because the data items necessary for inclusion in a combined analysis were not reported [662]. The data were extracted as %HbA1c and as units per day for insulin dose (using mean weight at baseline in each treatment group to convert insulin units per kg per day to units per day). For some studies, only attained mean levels were available rather

than changes from baseline by treatment group; therefore, treatment effect was derived as the net difference in absolute units of outcome between metformin and placebo groups. The obvious methodological heterogeneity in study design, drug dose, age of subjects, and length of follow up render the combined estimates of effect somewhat imprecise.

Section II - Results

4.3 Systematic review

The initial electronic search identified 187 studies (figure 4.1). A manual review of the citations yielded an additional ten studies. In total, 47 of these publications were judged to be relevant to metformin therapy in T1DM. Analysis of publications revealed: 17 were observational studies with no random allocation and/or no comparator group [656, 663-678]; 11 were reviews, letters or commentaries [679-689]; two did not contain any quantitative estimates of effects [690, 691]; one concerned an outcome (erythrocyte binding of insulin) not judged relevant [692]; and four were abstracts of later published papers [693-696]. Of the remaining 12 publications, one concerned insulin-requiring T2DM rather than T1DM (noted after translation) [697], and one covered a treatment period of less than seven days [698]. Only 10 studies were therefore identified [657, 659, 660, 662, 699-704]. Of these, one which was conducted on participants living in a children's home and did not mention informed consent, was excluded from further analysis [703].

The final nine studies [657, 659, 660, 662, 699-702, 704] covered a total of 192.8 patient years, and the number of completed subjects ranged from 10-92 (median 26) (two studies did not report number completed [660, 662] (Table 4.1). Total maximum daily metformin dose varied from 1000 mg to 2550 mg; duration of therapy ranged from 7 days to 12 months (median 4 months). Two studies were only available in abstract form [660, 662], including one of the largest studies (n = 80) which dated from 2000 [662].

All nine studies evaluated at least one parameter of glycaemic control or blood glucose in association with metformin treatment (table 4.1) but only seven reported mean change in HbA1 or HbA1c [657, 662, 699-702, 704], which was reduced by 0.6-0.9% in four studies [657, 662, 700, 701], with no significant change in three [699, 702, 704] (overall range +0.13% [699] to -0.9% [701]) (table 4.2). The remaining two (shorter term) studies reported other glycaemic benefits including an 18% increase in glucose uptake (artificial pancreas hyperinsulinaemic euglycaemic clamp) [659], and improved post-prandial glucose handling [660].

Of the seven studies in which insulin dose was not fixed by design) [657, 662, 699-702, 704], insulin dose requirement was reduced by 5.7-10.1 units/ day in six of seven studies (the study which reported no change was conducted in adolescents [701]. The same seven studies were of sufficient duration to report data on changes in weight or BMI. Metformin reduced weight by 1.7-6.0 kg in three [662, 699, 704] of six studies [657, 662, 699, 701, 702, 704]. A sustained and statistically significant reduction (mean 1.74 kg) was reported in the largest study, which was also of the longest duration [699] (table 4.2).

Figure 4.1 - Flow chart of the literature search

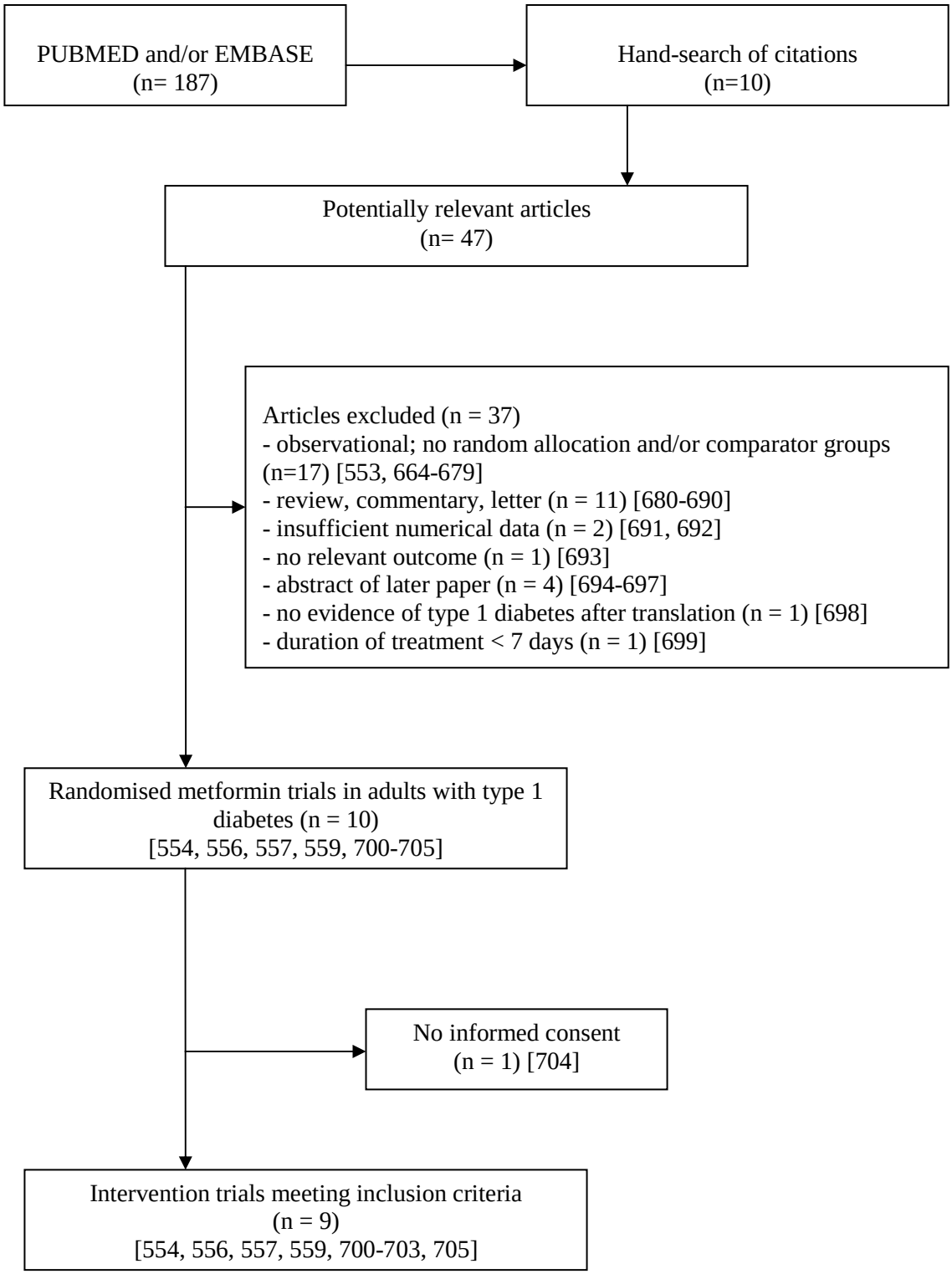


Table 4.1 - Study design and baseline characteristics of participants.

First author [reference]	*Year*	*Form of publication*	*Design*	*Random allocation sequence*	*Comparison group*	*Blinding of investigator /patient*	*Number of patients randomised (completed)*	*Duration in months (or as stated)*	*Mean age (years)*	*Mean weight (kg)*	*HbA1c (%) at baseline*	*Daily dose metformin (mg)*
Gin **[659]**	1985	Full	Crossover	[b]	Placebo	No /No	10 (10)	(7 days)	41	62	10.0[a]	1700
Keen **[660]**	1987	Abstract	Crossover	[b]	Placebo	Yes /Yes	8 ([b])	(3 weeks)	'Adults'[b]	84	[b]	1500
Walravens **[662]**	2000	Abstract	Parallel group	[b]	Placebo	Yes /Yes	80 ([b])	6	16	68	9.6	1000
Meyer **[702]**	2002	Full	Parallel [c] group	[b]	Placebo	Yes /Yes	62 (59)	6	41	76	7.6	1700
Hamilton **[700]**	2003	Full	Parallel group	Computer generated	Placebo	Yes /Yes	30 (27)	3	16	63 (MF), 71 (PL)	9.4 (MF), 8.9 (PL)	Up to 2000 (weight-dependent)
Särnblad **[701]**	2003	Full	Parallel group	[b]	Placebo	Yes /Yes	30 (26)[d]	3	17	68	9.3	Forced titration to 2000
Khan **[657]**	2006	Full	Crossover	Computer generated	Placebo	Yes /Yes	15 (15)	4	48	92	8.6	Forced titration to 2550
Lund **[699]**	2008	Full	Parallel [c] group	Computer generated	Placebo	Yes /Yes	100 (92)	12	46	80	9.5	Forced titration to 2000
Jacobsen **[704]**	2009	Full	Parallel group	[b]	Placebo	Yes /Yes	24 (23)	6	0	90	8.9 (MF) 9.3 (PL)	Forced titration to 2000

[a] HbA1; [b] Further data unavailable; [c] intention to treat analysis; [d] 24 completed the hyperinsulinaemic euglycaemic clamp procedure; MF, metformin; PL, placebo

Table 4.2 - Study outcomes

First author [reference]	*Year*	*Main outcome*	*Effect on %HbA1c*	*Effect on insulin dose*	*Effect on weight/ anthropometry*	*Other main effect(s)*	*No of hypoglycaemic events*	*Lipids*
Gin [659]	1985	Glucose uptake	[a]	Fixed by design (HEC with Biostator)	[a]	18% increase in insulin sensitivity ($p<0.01$)[b,c]	[a]	No significant differences with MF[b]
Keen [660]	1987	Fasting and postprandial glucose	Not measured (reduced mean 7 point capillary glucose -1.6[c] [MF] vs 0.1[c] [PL] mmol/L; $p<0.05$)	No change (fixed CSII)	No significant change[b]	No significant difference in change in fasting venous plasma glucose (-1.7[c] [MF] vs -0.9[c] [PL] mmol/L; *p*=NS)	7 (MF), 0 (PL); 'trend towards more hypos'; *p*=NS severity of events not specified	[a]
Walravens [662]	2000	HbA1c	0.7% lower with MF at 3 months ($p<0.05$); no difference at 6 months[c,d]	Reduced by 10% with MF in males at 6 months only[a]	Wt: MF 64 kg[d], PL 70 kg[d]; $p<0.05$ at 3 months WC: MF 74 cm[d], PL 77 cm[d]; $p<0.05$ at 3 months No significant effects at 6 months	[a]	[a]	HDL increased by 7 mmol/L[c,d] (22%) with MF (*p*='significant')[a]

Table 4.2 continued - Study outcomes

Author	*Year*	*Main outcome*	*Effect on %HbA_{1c}*	*Effect on insulin dose*	*Effect on weight/ anthropometry*	*Other main effect(s)*	*No of hypoglycaemic events*	*Lipids*
Meyer [702]	2002	Insulin dose (CSII)	No significant difference -0.13%[c] (MF) vs -0.11%[c] (PL) ('remained unchanged'[b])	6.0 fewer U per day[c] with MF compared with PL (p=0.0043)	No significant change[a]	4.5 fewer U[c] of basal insulin dose per day with MF compared with PL (p<0.023)	Minor: similar for MF and PL 47.2[c] (MF) vs 45.1[c] (PL) events patient^{-1} month^{-1} (p=NS) Major:19 (MF) vs 8 (PL) 'no significant difference'	MF: TC reduced by 0.41 mmol/L[c] (p=0.04); PL: no data[b]
Hamilton [700]	2003	Insulin sensitivity (FSIGT); HbA1c	0.6 %[c] lower with MF compared with PL (p=0.03)	0.16[c] U kg^{-1} day^{-1} lower with MF compared with PL (p=0.01)	'Trend towards lower BMI in MF group' -0.05[c] (MF) vs 0.2[c] (PL) kg/m^2 (p=NS)	No significant difference in the change in insulin sensitivity from baseline between MF and PL 2.6 ×10^{-4} min^{-1} μU^{-1} ml-^{1} (1.0-4.1)[e] (MF) vs 2.5 ×10^{-4} min^{-1} μU^{-1} ml^{-1} (1.9-2.9)[e] (PL) (p=NS)	Minor: 1.8[c] (MF) vs 0.9[c] (PL) events patient^{-1} week^{-1} (p=0.03) Major: 2 (MF), 1 (PL)	'No significant change'[d]
Särnblad [701]	2003	HbA_1c	0.9 % (-1.6, -0.1)[e] lower with MF (p<0.05)[b]	No significant change over time for either treatment group[b]	No significant change in wt 66 to 67 kg[c] (MF) 65 to 66 kg[c] (PL)[b] No significant change in BMI, WC or WHR[b]	Statistically significant (but variable) increase in insulin sensitivity from baseline with MF, not with placebo (HEC) (p<0.05)[b]	Minor[a] Major: none reported	'No significant change over time for either treatment group'[a]

Table 4.2 continued - Study outcomes

Author	*Year*	*Main outcome*	*Effect on %HbA_{1c}*	*Effect on insulin dose*	*Effect on weight/ anthropometry*	*Other main effect(s)*	*No of hypoglycaemic events*	*Lipids*
Khan [657]	2006	HbA1c	0.7 %[a] lower with MF compared with PL (p<0.005)	8 U[a] fewer per day with MF compared with PL (p<0.05)	-2 kg[c] (MF) vs -1 kg[c] (PL) (p=NS)	Fasting plasma glucose 4.3 mmol/L[c] lower with MF compared with PL (p<0.001)	Minor: 12 (MF) vs 11 (PL) episodes patient^{-1} 4 weeks^{-1} (p=NS) Major: 'none were reported'	TC and LDL lowered by 0.3 mmol/L[c] and 0.2 mmol/L[c], respectively, by MF (p=NS for the difference between MF and PL)
Lund [699]	2008	HbA1c	No significant effect with MF (0.13% [-0.19, 0.44][e]; p=NS)	5.7 U (-8.6, -2.9)[e] fewer per day with MF (p<0.001)	Wt reduced by 1.74 kg (-3.32,-0.17)[e] with MF compared with PL (p=0.03) BMI reduced by 0.56kg/m^2 (-1.06, -0.05)[e] with MF compared with PL (p=0.03) HC reduced by 2.90cm (-5.03, -0.77)[e] with MF compared with PL (p=0.008)	Significant reduction in cobalamin (-83.3 pmol/L [-139.3, -27.3][e]; p=0.004) and alkaline phosphatase (5.91 U l^{-1} [-10.77, -1.05][e]; p=0.018) from baseline with MF compared with PL Significant increase in potassium (0.20 mmol/L [0.02, 0.38][e]; p=0.029) with MF compared with PL	Minor: 48% of patients (MF) vs 49% of patients (PL) (not compared statistically) Major: 15% of patients (MF) vs 10% of patients (PL) (p=NS) Borderline increase in patients experiencing unconsciousness: 6% (MF) vs 1% (PL) (p=0.06) Major hypoglycaemic events leading to unconciousness during follow-up: 10 (MF) vs 2 (PL) (p<0.05)	Significant reductions in TC and LDL in MF-treated patients compared with PL[f] TC: -0.37 mmol/L (-0.67, -0.06)[e] (p=0.021) LDL: -0.33 mmol/L (-0.61, –0.06)[e] (p = 0.018)

Table 4.2 continued - Study outcomes

Author	*Year*	*Main outcome*	*Effect on %HbA_{1c}*	*Effect on insulin dose*	*Effect on weight/ anthropometry*	*Other main effect(s)*	*No of hypoglycaemic events*	*Lipids*
Jacobsen [704]	2009	HbA1c	No significant difference (-0.48[c] [MF] vs -0.17[c] (PL)%; p = NS)	8.8 U (-14.62, -3.04)[e] fewer per day with MF (p = 0.004)	Wt was 3.9 kg (-7.01, -0.71)[e] lower with MF compared with PL (p = 0.02)	No significant difference in systolic or diastolic blood pressure (daytime or night-time) compared with baseline or between treatment groups Comparing with baseline values: DSBP: -1.1[c] (MF) vs -4.2[c] (PL) mmHg (*p* = NS) DDBP: -2.4[c] (MF) vs -8.7[c] (PL) mmHg (*p* = NS) NSBP: -4.8[c] (MF) vs -0.4[c] (PL) mmHg (*p* = NS) NDBP: -4.5[c] (MF) vs 2.4[c] (PL) mmHg (p = NS)	[g] Significantly higher frequency with MF (0.7[c] [MF] vs 0.3[c] [PL] events patient^{-1} week^{-1} (*p*= 0.005]) 'the increased frequency was most distinct in the first 8 weeks'[a]	No significant differences in change in TC, LDL, between treatment groups[f] TC: -0.09 [c] (MF) vs 0.03 [c] (PL) mmol/L (*p* = 0.80) LDL: -0.23[c] (MF) vs -0.10[c] (PL) mmol/L (*p* = NS)

To convert values for insulin sensitivity to SI units (from $\times 10^{-4}$ min^{-1} $[pmol/L]^{-1}$) multiply by 0.167
[a]*Further data unavailable*
[b]*No p value reported for between-treatment comparison*
[c]*95% CI unavailable*
[d]*No variance estimates stated*
[e]*95% CI*
[f]*Lipid data published separately [705]*
[g]*Only biochemical hypoglycaemia was registered*
CSII, continuous subcutaneous insulin infusion; DDBP, daytime diastolic blood pressure; DSBP, daytime systolic blood pressure; FSIGT, frequently sampled intravenous glucose tolerance test; HC, hip circumference; HEC, hyperinsulinaemic–euglycaemic clamp; MF, metformin; NDBP, night-time diastolic blood pressure; NSBP, night-time systolic blood pressure; PL, placebo; TC, total cholesterol; WC, waist circumference; Wt, weigh

Total cholesterol was reported in seven studies: it was reduced by 0.37 mmol/L in comparison with placebo in the largest study [705], and by 0.3-0.41 mmol/L with respect to baseline (but not placebo) in two others [657, 702]. “No change” was reported in the other four studies [659, 700, 701, 704] (table 4.2).

4.4 Meta-analyses

For formal meta-analysis, only five studies reported the necessary means and standard deviations for insulin dose and HbA_{1c} [699-702, 704]; there were insufficient data for weight and lipids. Figures 4.2 to 4.5 summarise the data in standardised mean differences between treatment groups (i.e. the mean difference/ standard deviation of mean difference). Analysing for all five studies, the overall effect on $\%HbA_{1c}$ was a standardised mean difference between treatment groups of – 0.10 (i.e. 0.10 standardised units lower in the metformin group 95% CI: standardised mean difference reduction of – 0.36 to 0.15, p = 0.42). This translates into an absolute difference of 0.11 units lower $\%HbA_{1c}$ in the metformin than placebo groups (not statistically significant) (figure 4.2). As there was some suggestion of heterogeneity (p = 0.175), we carried out a sensitivity analysis of the four smaller and shorter studies [700-702, 704]. Thus, excluding the largest study [699] the overall effect on %HbA1c was a standardised mean difference between treatment groups of -0.30 (i.e. 0.30 standardised units lower in the metformin group 95% CI: standardised mean difference of -0.64 to 0.037, p = 0.081). This translates into an absolute difference of 0.28 units lower $\%HbA_{1c}$ (not statistically significant) in the metformin than placebo groups, with little evidence of heterogeneity (p = 0.353) (figure 4.3).

All five studies [699-702, 704] showed a reduction in daily insulin dose with metformin, with the overall measure of the treatment effect being a standardised mean difference between treatment groups of -0.65 (i.e. 0.65 standardised units lower in the metformin group 95% CI: standardised mean difference of – 0.92 to -0.39 units, $p < 0.001$). This translates into an absolute difference of 6.6 insulin units per day lower in the metformin than placebo groups. The chi-squared test of heterogeneity was not statistically significant ($p = 0.41$) with most of the information coming from the Lund et al.. study [699] (figure 4.4). A similar sensitivity analysis of the four smaller and shorter studies [700-702, 704], excluding Lund et al. [699] confirmed a reduction in daily insulin dose with metformin, with the overall measure of the treatment effect being a standardised mean difference between treatment groups of -0.55 (i.e. 0.55 standardised units lower in the metformin group 95% CI: standardised mean difference of – 0.90 to -0.21 units, $p = 0.002$). This translates into an absolute difference of 7.16 insulin units per day lower in the metformin than placebo groups. The chi-squared test of heterogeneity was not statistically significant ($p = 0.365$) with most of the information coming from Meyer et al.. [702] (figure 4.5).

There were trends for increased major and/or minor hypoglycaemia with metformin therapy in six [657, 660, 699, 700, 702, 704] out of seven studies in which this adverse effect was mentioned [657, 660, 699-702, 704] (table 4.2); this reached statistical significance in two of the smaller studies [700, 704]. There were no reports of lactic acidosis associated with metformin therapy. Rates of gastrointestinal adverse effects were not systematically reported except in two studies [699, 704],

with rates being nearly identical in metformin and placebo groups in the largest study [699],

No studies of any design evaluating cardiovascular function, structure or events were identified.

4.5 Discussion

This study found only nine randomised studies of metformin therapy in T1DM, two of which were small and experimental. There were only 192.8 patient years of randomised follow-up in the literature which compares adversely with the evidence for statin therapy in T1DM (over 6000 patient years), although even this is inconclusive [706]. Reflecting the paucity of the evidence underpinning metformin in T1DM, recent publication of a single study [699] from the Steno Diabetes Centre almost doubled the available patient years of randomised follow-up. Overall the grade of evidence according to the Cochrane GRADE system for the main outcomes of glycaemic control and insulin dose is at best 'moderate' [658].

Only five studies [699-702, 704] could be formally combined in a meta-analysis: there are obvious constraints to the interpretations of such sparse and heterogeneous data. Nevertheless, there was evidence of a significant effect of metformin in reducing daily insulin dose requirement. Overall, the evidence reviewed in this study is consistent with a whole-body insulin-sensitising effect of metformin. A predicted concomitant attenuation in weight gain with lowering of required insulin doses was seen in the largest and longest trial [699], which was twice the duration of any other

Figure 4.2 - Standardised mean difference of HbA1c level between metformin-treated and metformin free type 1 diabetes patients for five randomised controlled studies, including the largest study to date [699] (see text for equivalent %HbA1c units)

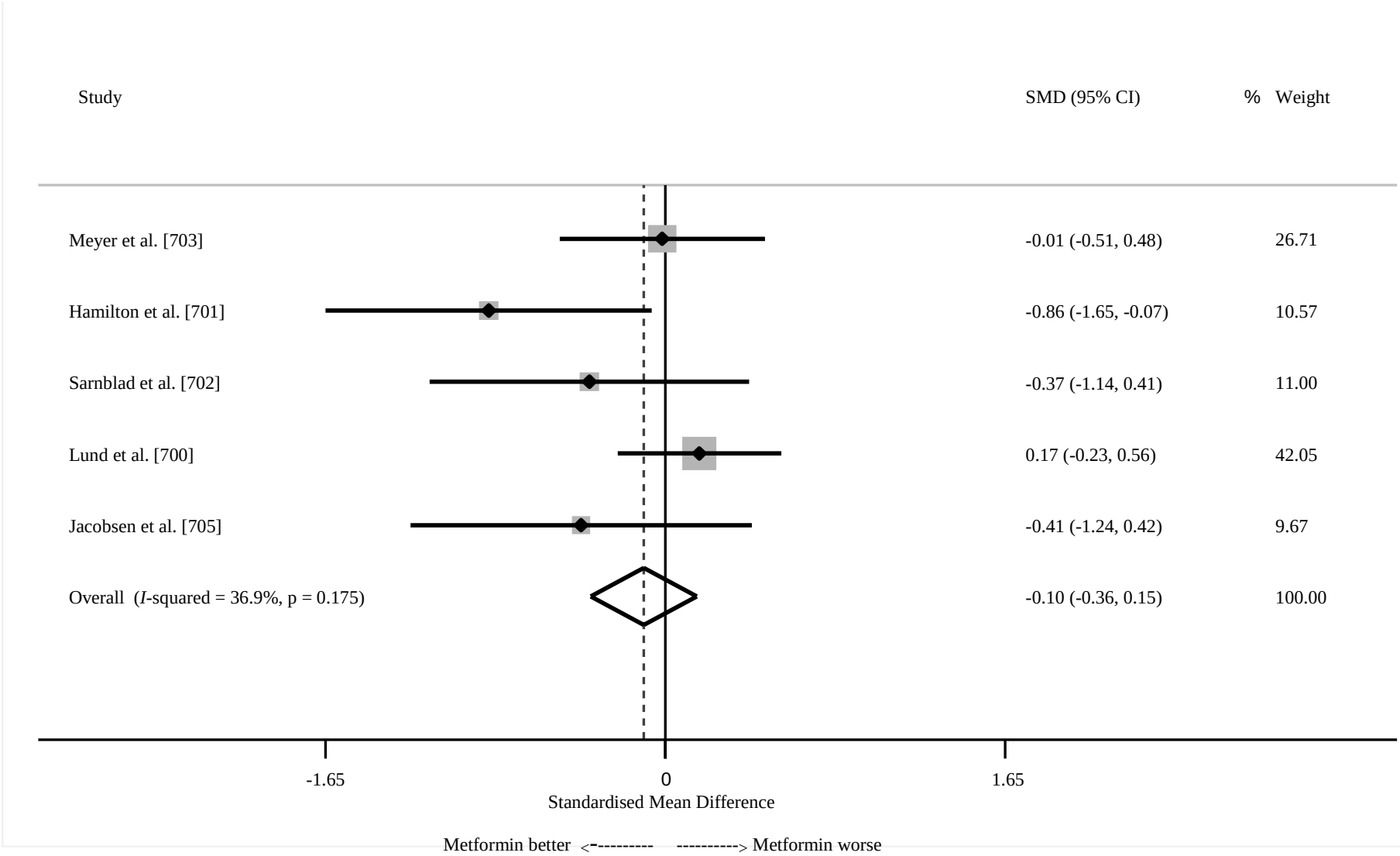


Figure 4.3 - Standardised mean difference of HbA1c level between metformin-treated and metformin free type 1 diabetes patients for four randomised controlled studies, excluding the largest study to date [699] (see text for equivalent %HbA1c units)

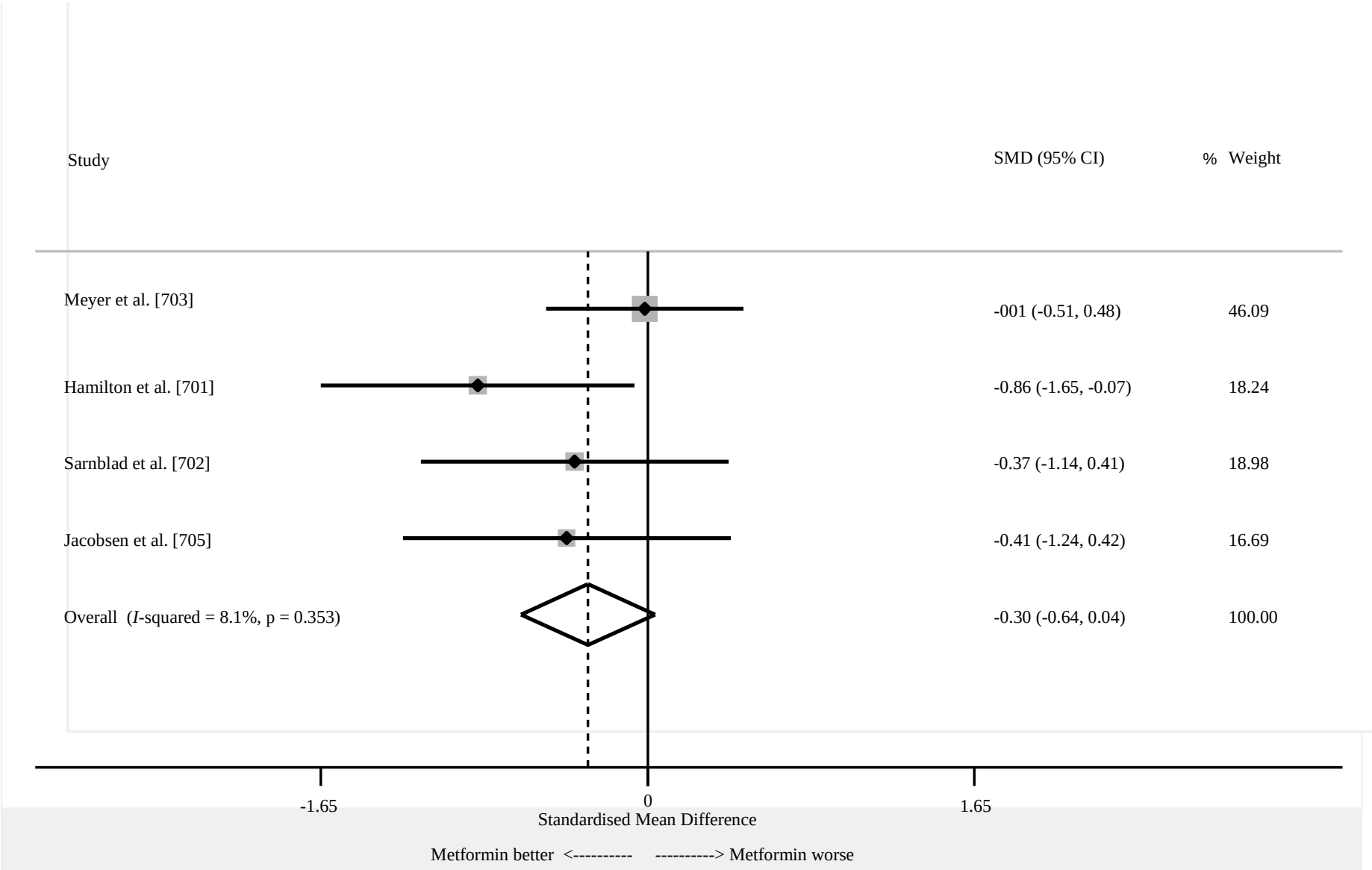


Figure 4.4 - Standardised mean difference of insulin dose between metformin-treated and metformin free type 1 diabetes patients for five randomised controlled studies, including the largest study to date [699] (see text for equivalent insulin dose units)

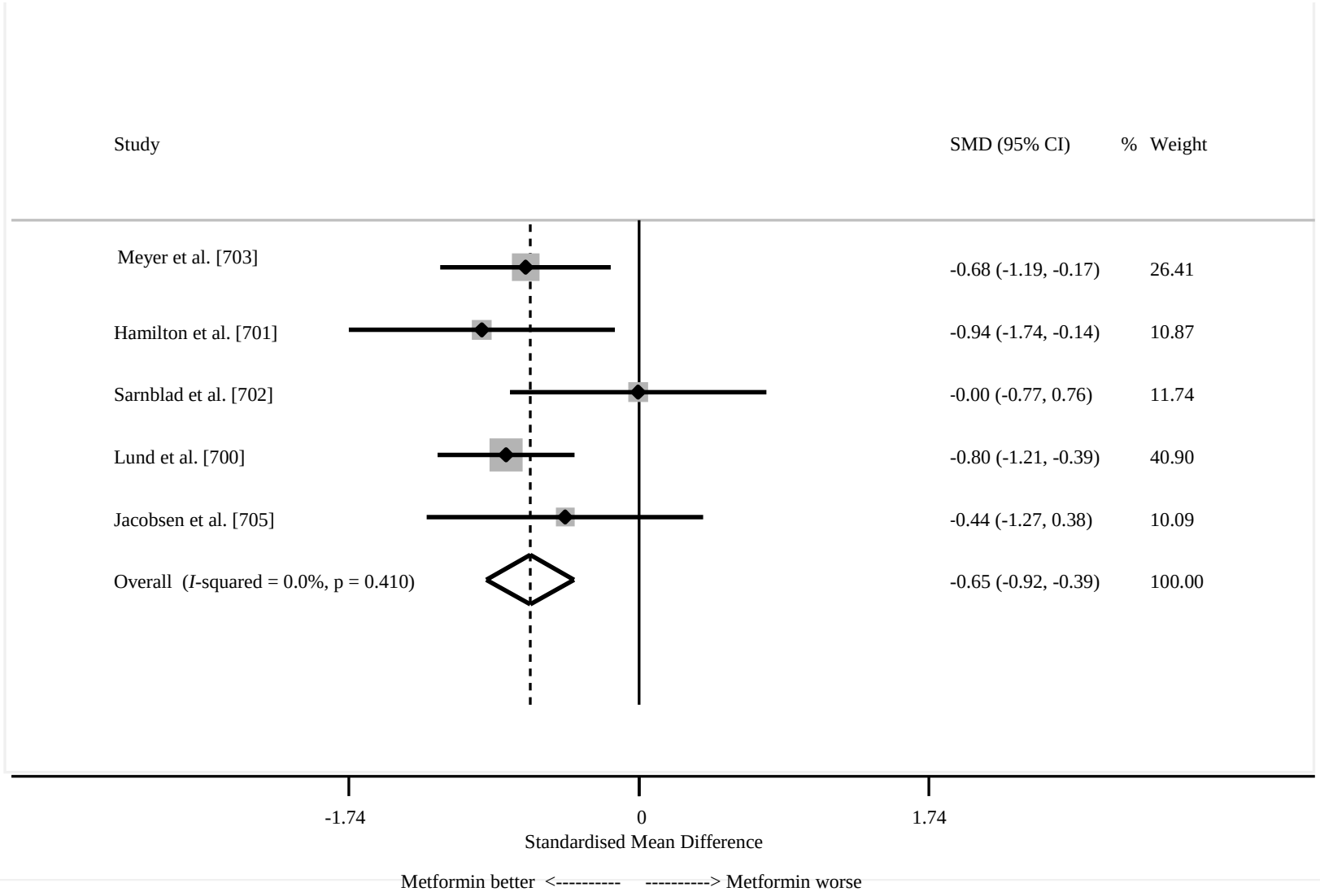
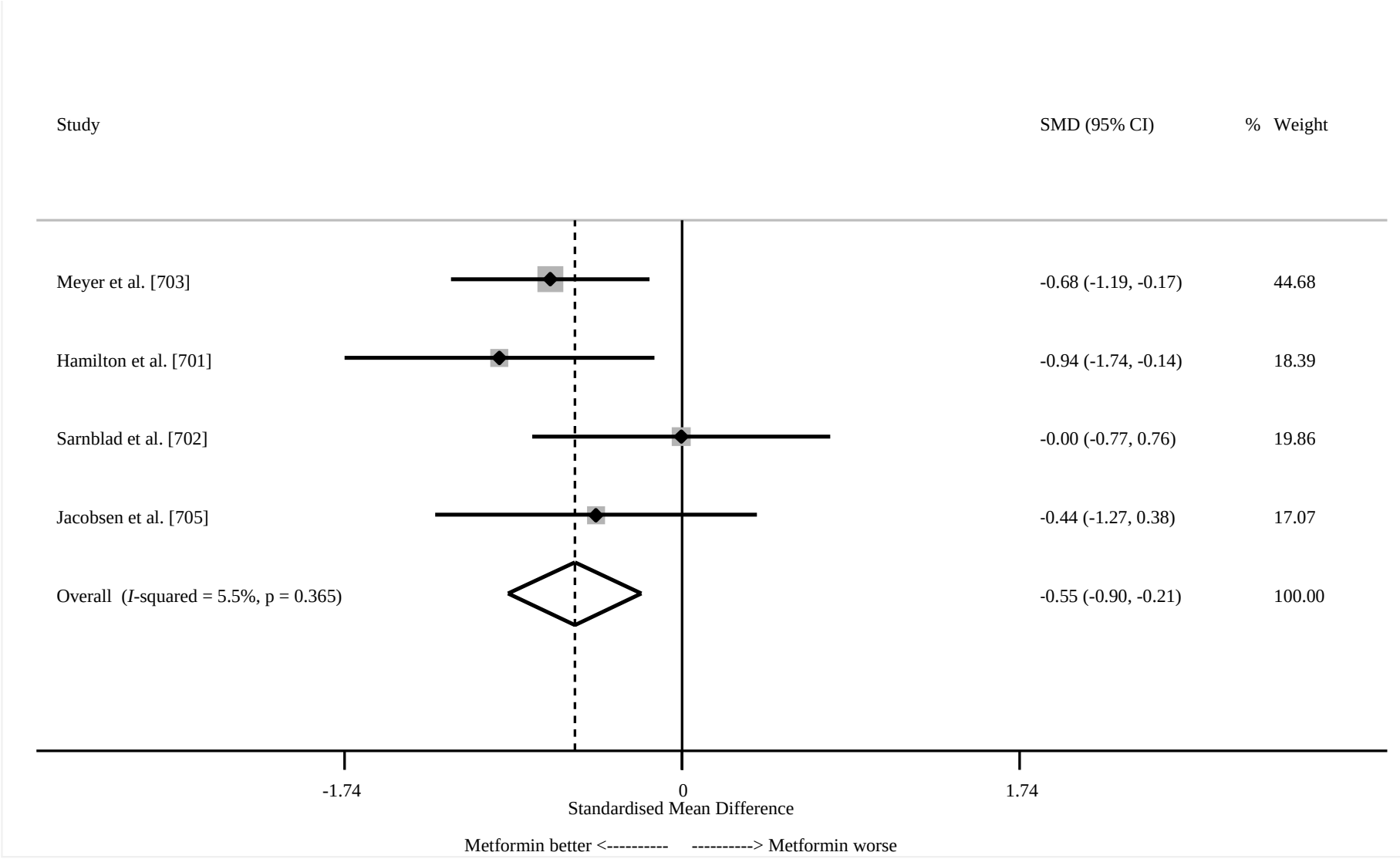


Figure 4.5 - Standardised mean difference of insulin dose between metformin-treated and metformin free type 1 diabetes patients for four randomised controlled studies, excluding the largest study to date [699] (see text for equivalent insulin dose units)



study. A reduction in weight was also reported over six months' treatment in the most recently-published study [704], in which use of a specific algorithm for insulin titration resulted in a mean dose reduction of 20%. In keeping with the evidence in T2DM, as recently reviewed by Wulffele et al. [607], there was also a relatively consistent signal that metformin may reduce total and LDL cholesterol in adults with T1DM [705].

In terms of adverse effects, this study noted trends towards increased rates of hypoglycaemia in association with adjunct metformin therapy, although this reached statistical significance in only two of the smaller trials [700, 704]. Furthermore, although the largest trial did not report increased rates of metformin-associated major or minor hypoglycaemia, there were significantly more major hypoglycaemic events leading to unconciousness among metformin-treated T1DM individuals [699]. Clearly, even with this weak evidence, physicians contemplating a recommendation of metformin therapy for their patients with T1DM should advise them carefully regarding insulin dose adjustment and blood glucose monitoring. Surprisingly, gastrointestinal adverse effects were infrequently mentioned by investigators. In the largest trial, two of 108 patients screened dropped out for this reason in a run-in period; thereafter, these effects occurred in almost half of the remaining patients, but in almost exactly equal proportions in the active and placebo groups [699]. No cases of lactic acidosis were reported in any of the trials. Although evidence from a Cochrane review has been reassuring on this account in T2DM [707], randomised follow-up is clearly insufficient in T1DM, and concern continues to be expressed by some physicians [682].

The findings of the present review disagree to some extent from those of another recent review [708]. Pang and Narendran reported a reduction in HbA_{1c} with metformin therapy in T1DM on the basis of their meta-analysis of the three smaller trials on this topic [657, 700, 701] which they chose to combine with one of the three larger trials [702], (but not the two largest [662, 699]), along with an observational (controlled but non-randomised) trial which did not meet this study's inclusion criteria [663]. At the time of their review, the largest trial [699] was only available in abstract form [696]. Thus, although this review has the limitation of being based on only 192.8 patient years of follow up, it is a significant advance on the 54 patient years available in the only comparable publication to date. The conclusions of both reviews on outcomes other than HbA_{1c} (weight reduction, insulin dose requirement and cholesterol) were, however, generally similar. While acknowledging that studies as short as one to three weeks are unlikely to yield information on efficacy, this review opted to include them simply as potential sources of information on safety and tolerability, particularly given the paucity of evidence available. These studies were excluded from the formal meta-analysis.

As potential chance differences (randomisation error) at baseline between groups allocated to treatment can influence the outcome of smaller studies, an ideal approach for meta-analysis is to base calculations on data adjusted for baseline values. As such information was not available for all studies, this study derived the treatment effects reported from absolute units of outcome; one acknowledges this as a limitation, but believe it unlikely to have significantly impacted on the conclusions. A further constraint is that magnitude of treatment effect can be influenced by differences in entry criteria between trials (e.g. for HbA1c): I believe that such

methodological issues inherent to meta-analysis only strengthen the case for further larger trials.

Following UKPDS [36] and its more recent 10-year post-randomisation follow-up [489], metformin is widely-considered to protect against cardiovascular complications in T2DM, which is the principal reason for its current status as first line therapy in this condition. It should be recalled that only 753 patients were included in this specific UKPDS randomisation, and that an effect in the other direction was observed when it was combined with a sulphonylurea [36, 164]. Recently published results from the HOME-trial have shown that metformin improves macrovascular outcomes in insulin-treated T2DM patients [493]. This is consistent with some data that metformin may have intrinsic (and possibly direct) beneficial effects independent of glucose-lowering on the cardiovascular system via activation of AMPK [709-711] in a number of conditions [709, 712, 713]. If this is accepted, the hypothesis that metformin might prevent cardiovascular complications in T1DM should also be tested formally, as even young adults with this condition have an extremely high relative risk of cardiovascular disease [714-716]. The data reviewed herein provides useful information to guide the design of such a future trial.

At the time of publication of this systematic review and meta-analysis, metformin therapy was not advocated in any major national or international guidelines for the management of T1DM, nor in Tayside's own regional guidelines. However, routine searches the authors recently conducted of anonymised T1DM prescription data in Tayside, Scotland [437] (population 400,000; ≅1850 classified as having T1DM and

diagnosed aged < 35 years), estimated that 7.9% with BMI > 27 kg/m^2 were receiving this medication, rising to 13.0% for those with BMI > 30 kg/m$^{2.}$ Even allowing for any residual misclassification, it is therefore likely that many thousands of people with T1DM worldwide are receiving an unproven therapy of unknown long-term efficacy (albeit a familiar one with an attractive theoretical underpinning and the potential to result in reductions in rates of cardiovascular disease). Considering that T1DM is usually diagnosed in childhood or adolescence and is a lifelong condition, I believe that properly-designed randomised controlled clinical trials of sufficient size and duration to have the power to show reductions in cardiovascular disease should be conducted forthwith. Given that metformin use in T2DM has also been associated with reduced cancer risk [717], it would additionally be desirable to investigate this relationship in metformin-treated people with T1DM.

Since the publication of this systematic review and meta-analysis, Burchardt et al. published the results of a prospective pilot clinical study of 33 obese young intensively-treated T1DM patients randomised to additional treatment with metformin for six months (vs 19 patients treated with insulin alone) [718]. The authors concluded that adjunct metformin was associated with a reduction in HbA1c (1.3%), fasting plasma glucose (3.10 mmol/L), post-prandial plasma glucose (3.59 mmol/L), average daily plasma glycaemia (1.62 mmol/L), triglycerides (0.24 mmol/L), glycated-LDL-cholesterol (0.02 mmol/L) and BMI (0.6 kg/m^2), albeit no significant changes in total cholesterol, LDL-cholesterol, oxidized LDL cholesterol and HDL cholesterol levels. Such differences were not reported among patients treated with insulin alone [718]. This study was however limited by a small sample

size, high drop-out rate (an additional 16 randomised patients did not complete the study) and open-label design.

In line with the results of this systematic review and meta-analysis, a recently published prospective pilot study of 42 uncomplicated T1DM patients [mean (SD) age = 46 (8) years for the metformin group; 41 (10) years for placebo] reported that use of adjunct metformin for six months improved flow mediated dilation (a surrogate marker of endothelial function/atherosclerosis) by 1.32% (95% CI 0.30, 2.43) and increased urinary 8-iso-prostaglandin F2α (a biomarker of oxidative stress) by 149 pg/mg creatinine (95% CI 50, 248), irrespective of its effects on body weight and glycaemic control [719]. It is hoped that the REducing with MetfOrmin Vascular Adverse Lesions in type 1 diabetes (REMOVAL) study, a phase III prospective trial currently recruiting 500 T1DM patients, will yield much-needed definitive data on the impact of adjunct metformin on common carotid artery intima media thickness (another surrogate marker of atherosclerosis), endothelial function, glycaemic control, insulin dose, weight, LDL-cholesterol, renal function (change in albuminuria and estimated glomerular filtration rate) and change in retinopathy stage [720].

In summary, this systematic review and meta-analysis of the randomised trials in the literature indicates that metformin therapy in T1DM is associated with a reduced insulin dose requirement but no clear evidence of an improvement in glycaemic control. In addition, there may be small reductions in weight and total/LDL-cholesterol, but there are no data on cardiovascular outcomes or their surrogates. This thesis' data suggest this is an important area for future study.

Chapter 5

Conclusions and future work

Chapter 5 - Conclusions and future work

This thesis set out to examine mechanisms underlying intolerance to thiazolidinedione therapy but concluded that oedema and heart failure as a clear consequence of thiazolidinedione therapy was less common than anticipated. Thiazolidinedione therapy was apparently less significant as a risk factor for oedema/HF than other common patient characteristics shared across first and second line oral glucose lowering agents (including metformin - sulphonylurea combination therapy). Thus, the reported association between thiazolidinedione therapy and oedema/HF may have been over-emphasised.

The population-based approach I employed permitted the identification of significant time-varying risk factors, notably macrovascular disease, alanine aminotransferase (ALT) and serum albumin. To my knowledge, such time-dependent risk variation pertinent to thiazolidinedone or metformin-sulphonylurea combination therapy has not been reported in the literature. Macrovascular disease consistently emerged as the strongest predictive factor for the adverse events of interest, with its relative contribution being highest in the first three to six months following thiazolidinedone or metformin-sulphonylurea prescription.

The relative infrequency of incident loop diuretic prescription (4.3%) and incident HF events (1.1%) following index thiazolidinedione therapy are consistent with the difficulties encountered identifying suitable patients fitting strict inclusion criteria for the exploratory, case-control study. Nonetheless, the latter renders the resulting cohort of thiazolidinedione tolerant subjects particularly valuable in research terms,

and permitted a novel comprehensive, albeit exploratory, physiological characterisation of such patients. Limited exploratory data from the two thiazolidinedione intolerant patients failed to suggest a role for VEGF during either acute or chronic 'high normal' salt loading. However, renin (and possibly aldosterone) appeared to reduce in these patients beyond the boundaries of reference intervals derived from their TZD-tolerant counterparts in this context. Moreover, concentrations of ANP (and possibly BNP) increased to a greater extent following chronic sodium exposure in these patients.

No echocardiographic differences were detected between the thiazolidinedione tolerant and intolerant subgroups, but haematocrit and DBP fell in the latter to a greater extent in response to salt loading, while cAI and pAI rose, suggesting that patients prone to thiazolidinedone-associated fluid retention may be characterised by a higher degree of ventricular-arterial stiffening in response to salt loading.

The systematic review and meta-analysis of publications investigating a role for adjunct metformin in T1DM underscored the paucity of data in this field, despite the fact that this commonly prescribed, cheap and effective first line oral glucose lowering agent is frequently prescribed to T1DM patients, particularly those at higher BMI ranges. A formal meta-analysis reported that use of adjunct metformin translates into a reduction in daily insulin dose requirements (6.6 units/day), despite no improvements in glycaemic control, possibly as T1DM patients tend to self-titrate their insulin dose towards their usual HbA1c. Adjunct metformin was generally well tolerated, with few reports of gastrointestinal upset and no evidence of lactic acidosis, albeit an increased tendency for hypoglycaemia. None of the available

studies reported cardiovascular outcomes. This thesis' published systematic review and meta-analysis [721] supported the successful grant application for the (currently recruiting) REducing With MetfOrmin Vascular Adverse Lesions in Type 1 Diabetes (REMOVAL) trial, an ongoing prospective, randomised clinical trial investigating the potential benefits of adjunct metformin in T1DM over three years [720].

The work described in this thesis highlights the unanticipated difficulties that can be encountered when attempting to recruit patients fitting strict inclusion criteria. However, the detailed characterisation of those TZD 'tolerant' and 'intolerant' patients that could be enrolled provides some information on the characteristics of patients who may be lower risk for adverse effects; it may also help to guide research aimed at designing modified agents with a better profile. I would be particularly interested to pursue further research in this field, recruiting patients from a larger catchment area. It may be prudent to subdivide the clinical study into multiple small studies with targeted inclusion and exclusion criteria pertinent to the specific measurements being made, so as to maximise patient recruitment without compromising on study quality. It would also be wise to repeat the population based study on a larger cohort of patients (possibly nation-wide), so as to validate the (unexpected) results arising from this Tayside cohort, and possibly allow the inclusion of a larger number of covariates in multivariate logistic and Cox regression models.

Recent results arising from the SAVOR-TIMI trial have alerted clinicians on a possible causal relationship between dipeptidyl peptidase-IV (DPP-IV) inhibitors and incident heart failure [722]. My thesis' population-based study validated index

loop diuretic prescription as a surrogate marker of fluid retention and heart failure. Such an approach could prove useful in the setting of DPP-IV inhibitor therapy (and other 'novel' glucose lowering agents), particularly as none of the available prospective clinical trials was specifically designed to investigate this adverse event.

In summary, as new pathways underpinning insulin signalling and insulin resistance are unravelled, there is likely to be renewed interest in new pharmacological insulin sensitizing agents to improve glycaemic control. A better understanding of licensed agents regarded as insulin sensitizers (metformin and thiazolidinediones) should provide beneficial insights in this regard. Published data arising from this thesis imply a potential advantageous role for adjunct metformin in T1DM, and should serve as a catalyst for large scale prospective research in this field. The association between thiazolidinediones and fluid retention/HF remains incompletely understood, and may have been over-emphasized. Population and clinical data suggest that careful prescribing practices, such as avoiding patients with known macrovascular disease, high BMI or raised alanine aminotransferase (ALT) may reduce the risk of adverse events in patients at risk, without removing a therapy with considerable efficacy from the glucose-lowering armamentarium.

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Appendix

Appendix

Table II.1 - Left ventricular ejection fraction (LVEF) measurements (%) and derived % differences between sodium load exposures for visits 2 and 3.

Subject number by category	*LVEF (%) (low Na)*	*LVEF (%) (acute high Na)*	*LVEF (%) (chronic high Na)*	*% difference LVEF (acute high Na - low sodium)*	*% difference LVEF (chronic high Na - low sodium)*
TZD tolerant					
1	55.3	[a]	57.0		3.1
2	68.0	68.0	66.0	0.0	-2.9
3	45.0	52.0	45.0	15.6	0.0
4	55.0	56.0	63.0	1.8	14.5
5	72.0	67.0	72.0	-6.9	0.0
6	67.3	62.3	58.0	-7.4	-13.8
7	58.0	60.0	63.0	3.4	8.6
8	63.0	66.0	63.0	4.8	0.0
9	55.0	55.0	64.0	0.0	16.4
Mean	**59.8**	**60.8**	**61.2**	**0.4**	**2.9**
(95% CI)	**(54.3, 65.3)**	**(56.6, 65.0)**	**(56.3, 66.1)**	**(3.2, -2.4)**	**(-3.2, 9.0)**
TZD intolerant					
10	67.0	62.0	62.0	-7.5	-7.5
11	64.0	69.0	63.0	7.8	-1.6

[a] ***Patient declined echocardiographic assessment on this occasion.***

Table II.2 - E-wave/A-wave (E/A) ratio readings and derived % differences between sodium load exposures for visits 2 and 3.

Subject number by category	*E/A ratio (low Na)*	*E/A ratio (acute high Na)*	*E/A ratio (chronic high Na)*	*% difference E/A ratio (acute high Na - low sodium)*	*% difference E/A ratio (chronic high Na - low sodium)*
TZD tolerant					
1	0.8	[a]	0.9		12.5
2	1.2	1.1	1.3	-8.3	8.3
3	0.9	0.8	0.9	-11.1	0.0
4	1.0	1.1	0.8	10.0	-20.0
5	1.0	0.9	0.8	-10.0	-20.0
6	0.7	1.1	0.8	57.1	14.3
7	0.7	0.9	0.7	28.6	0.0
8	0.6	0.6	0.7	0.0	16.7
9	0.7	0.7	0.9	0.0	28.6
Mean	**0.8**	**0.9**	**0.9**	**8.3**	**4.5**
(95% CI)	**(0.67, 0.93)**	**(0.76, 1.04)**	**(0.77, 1.03)**	**(-8.1, 24.7)**	**(-6.2, 15.2)**
TZD intolerant					
10	1.0	1.0	0.9	0.0	-10.0
11	0.7	0.7	1.2	0.0	71.4

[a] ***Patient declined echocardiographic assessment on this occasion.***

Table II.3 - E prime (E') readings and derived % differences between sodium load exposures for visits 2 and 3.

Subject number by category	*E prime (low Na)*	*E prime (acute high Na)*	*E prime (chronic high Na)*	*% difference E prime (acute high Na - low Na)*	*% difference E prime (chronic high Na - low Na)*
TZD tolerant					
1	5.70	[a]	6.82	[b]	19.6
2	4.19	4.87	4.90	16.2	16.9
3	4.97	5.17	5.19	4.0	4.4
4	6.20	6.70	6.82	8.1	10.0
5	9.07	7.50	5.10	-17.3	-43.8
6	6.80	6.60	6.90	-2.9	1.5
7	5.56	5.07	5.07	-8.8	-8.8
8	3.51	4.39	4.58	25.1	30.5
9	4.09	3.61	4.87	-11.7	19.1
Mean	**5.60**	**5.50**	**5.60**	**1.6**	**5.5**
(95% CI)	**(4.50, 6.70)**	**(4.60, 6.40)**	**(4.90, 6.30)**	**(-8.5, 11.7)**	**(-8.7, 19.7)**
TZD intolerant					
10	5.46	5.95	5.95	9.0	9.0
11	5.17	5.07	5.20	-1.9	0.6

[a] Patient declined echocardiographic assessment on this occasion.; [b]calculation not possible due to missing data

Table II.4 - E wave/E prime (E/e') ratio readings and derived % differences between sodium load exposures for visits 2 and 3.

Subject number by category	*E/e' (low Na)*	*E/e' (acute high Na)*	*E/e' (chronic high Na)*	*% difference E/e' (acute high Na - low Na)*	*% difference E/e' (chronic high Na - low Na)*
TZD tolerant					
1	8.4	[a]	8.3	[b]	-1.2
2	19.7	17.8	18.5	-9.6	-6.1
3	12.9	9.4	9.3	-27.1	-27.9
4	11.1	11.7	9.1	5.4	-18.0
5	7.2	10.3	11.8	43.1	63.9
6	11.3	15.4	9.0	36.3	-20.4
7	11.3	14.7	11.3	30.1	0.0
8	20.1	16.3	11.1	-18.9	-44.8
9	13.5	14.8	13.1	9.6	-3.0
Mean	**12.8**	**13.8**	**11.3**	**8.61**	**-6.39**
(95% CI)	**(9.86, 15.74)**	**(11.72, 15.88)**	**(9.27, 13.33)**	**(-9.55,26.77)**	**(26.09,13.31)**
TZD intolerant					
10	16	15.8	16.4	-1.3	2.5
11	7.2	8.3	11	15.3	52.8

[a] Patient declined echocardiographic assessment on this occasion; [b] calculation not possible due to missing data

Table II.5 - Left ventricular mass (LVM) readings (g) and derived % differences between sodium load exposures for visits 2 and 3.

Subject number by category	*LVM (g) (low Na)*	*LVM (g) (acute high Na)*	*LVM (g) (chronic high Na)*	*% difference LVM (g) (acute high Na - low Na)*	*% difference LVM (g) (chronic high Na - low Na)*
TZD tolerant					
1	220.0	[a]	221.0	[b]	0.5
2	241.2	240.0	242.0	-0.5	0.3
3	257.0	257.0	260.0	0.0	1.2
4	194.0	194.0	206.0	0.0	6.2
5	150.0	150.0	150.0	0.0	0.0
6	170.0	170.0	170.0	0.0	0.0
7	237.0	237.0	235.0	0.0	-0.8
8	263.0	263.0	269.0	0.0	2.3
9	251.0	251.0	251.9	0.0	0.4
Mean	**220.4**	**220.3**	**222.8**	**-0.06**	**1.12**
(95% CI)	**(194.1, 246.7)**	**(190.5, 250.1)**	**(196.2, 249.4)**	**(-0.20, 0.08)**	**(-0.25, 2.49)**
TZD intolerant					
10	175.0	176.0	175.6	0.6	0.3
11	198.0	198.0	198.0	0.0	0.0

[a] Patient declined echocardiographic assessment on this occasion; [b] calculation not possible due to missing data.

Table II.6 - Vascular endothelial growth factor (VEGF) measurements (pg/mL) and derived % differences between sodium load exposures for visits 2 and 3.

Subject number by category	*VEGF (pg/mL) (low sodium)*	*VEGF (pg/mL) (chronic high sodium)*	*% difference VEGF (chronic high sodium - low sodium)*
TZD tolerant			
1	42.8	39.8	-7.0
2	27.7	42	51.6
3	21.7	19.1	-12.0
4	39	31.1	-20.3
5	217.7	94.8	-56.5
6	30.4	28.3	-6.9
7	47.4	34.7	-26.8
8	31.8	27	-15.1
9	[a]	30.4	[b]
Mean	**57.3**	**38.6**	**-11.6**
(95% CI)	**(12.0, 102.6)**	**(24.1, 53.1)**	**(-32.53, 9.33)**
TZD intolerant			
10	21	25.6	21.9
11	33.2	25.2	-24.1

[a] Patient's VEGF data unavailable; [b]derivation of % difference not possible due to missing data

Table II.7 - Plasma copeptin measurements (pmol/L) and derived % differences between sodium load exposures for visits 2 and 3

Subject number by category	*Copeptin (pmol/L) (low sodium)*	*Copeptin (pmol/L) (chronic high sodium)*	*% difference copeptin (chronic high sodium - low sodium)*
TZD tolerant			
1	4.80	4.20	-12.5
2	6.13	6.70	9.3
3	9.19	5.26	-42.8
4	9.20	4.31	-53.2
5	1.22	1.76	44.3
6	4.30	3.72	-13.5
7	3.00	2.47	-17.7
8	8.78	4.27	-51.4
9	[a]	4.36	[b]
Mean	**5.83**	**4.10**	**-17.2**
(95% CI)	**(3.73, 7.93)**	**(3.19, 5.01)**	**(-40.87, 5.67)**
TZD intolerant			
10	1.81	1.26	-30.4
11	9.57	10.29	7.5

[a] patient's plasma copeptin data were unavailable; [b] estimation of % difference not possible due to missing data

Table II.8 - Systolic blood pressure (SBP) readings (mmHg) and derived % differences between sodium load exposures for visits 2 and 3.

Subject number by category	*SBP (mmHg) (low sodium)*	*SBP (mmHg) (chronic high sodium)*	*% difference SBP (chronic high sodium - low sodium)*
TZD tolerant			
1	119.0	132.3	11.2
2	149.0	150.0	0.7
3	143.3	134.3	-6.3
4	139.7	143.7	2.9
5	132.7	127.3	-4.0
6	148.7	148.3	-0.2
7	131.7	119.3	-9.4
8	145.3	135.0	-7.1
9	138.0	153.0	10.9
Mean	**138.6**	**138.1**	**-0.2**
(95% CI)	**(132.3, 144.9)**	**(130.7, 145.5)**	**(-5.1, 4.7)**
TZD intolerant			
10	157.3	134.7	-14.4
11	141.0	158.3	12.3

Table II.9 - Mean arterial pressure (MAP) readings (mmHg) and derived % differences between sodium load exposures for visits 2 and 3.

Subject number by category	*MAP (mmHg) (low sodium)*	*MAP (mmHg) (chronic high sodium)*	*% difference MAP (chronic high sodium - low sodium)*
TZD tolerant			
1	95.9	103.9	8.3
2	99.0	98.7	-0.3
3	98.9	100.8	1.9
4	107.2	109.2	1.9
5	94.7	89.6	-5.4
6	108.2	107.4	-0.7
7	102.1	93.1	-8.8
8	109.8	103.7	-5.6
9	100.4	107.0	6.5
Mean	**101.8**	**101.5**	**-0.2**
(95% CI)	**(98.2, 105.4)**	**(97.1, 105.8)**	**(-3.9, 3.5)**
TZD intolerant			
10	114. 7	102.4	-10.7
11	109.4	112.3	2.6

Appendix Table III.1 - Unadjusted odds ratio of index loop diuretic prescription after exposure to index insulin therapy (vs thiazolidinedione therapy)

Gender status	*Unadjusted odds ratio of index loop diuretic prescription following exposure to insulin (vs thiazolidinedione therapy)*	*95% confidence intervals*	
		Lower	*Upper*
Males and females	3.18	2.44	4.15
Males	3.74	2.53	5.52
Females	2.64	1.84	3.80

Appendix table III.2 - Unadjusted odds ratio of index loop diuretic prescription after exposure to index insulin therapy (vs metformin-sulphonylurea combination therapy)

Gender status	*Unadjusted odds ratio of index loop diuretic prescription following exposure to insulin (vs metformin-sulphonylurea combination therapy)*	*95% confidence intervals*	
		Lower	*Upper*
Males and females	2.89	2.28	3.67
Males	2.58	1.86	3.58
Females	3.24	2.28	4.58

Appendix table III.3 - Unadjusted odds ratio of index loop diuretic prescription after exposure to index thiazolidinedione therapy (vs metformin-sulphonylurea therapy)

Gender status	*Unadjsuted odds ratio of index loop diuretic prescription following exposure to thiazolidinediones (vs metformin-sulphonylurea combination therapy)*	*95% confidence intervals*	
		Lower	*Upper*
Males and females	0.91	0.69	1.20
Males	0.69	0.47	1.02
Females	1.23	0.83	1.81

Appendix table III.4 - Unadjusted odds ratio of incident heart failure after exposure to index insulin therapy (vs thiazolidinedione therapy)

Gender status	*Unadjusted odds ratio for incident heart failure following exposure to insulin (vs thiazolidinedione therapy)*	*95% confidence intervals*	
		Lower	*Upper*
Males and females	3.24	2.07	5.07
Males	2.93	1.64	5.26
Females	3.83	1.87	7.85

Appendix table III.5 - Unadjusted odds ratio of incident heart failure after exposure to index insulin therapy (vs metformin-sulphonylurea combination therapy)

Gender status	*Unadjusted odds ratio of incident heart failure following exposure to insulin (vs metformin-sulphonylurea combination therapy)*	*95% confidence intervals*	
		Lower	*Upper*
Males and females	2.52	1.72	3.67
Males	2.14	1.31	3.50
Females	3.32	1.81	6.11

Appendix table III.6 - Unadjusted odds ratio of incident heart failure after exposure to index thiazolidinedione therapy vs metformin-sulphonylurea therapy

Gender status	*Unadjusted odds ratio of incident heart failure following exposure to thiazolidinediones (vs metformin-sulphonylurea combination therapy)*	*95% confidence intervals*	
		Lower	*Upper*
Males and females	0.78	0.49	1.24
Males	0.73	0.41	1.30
Females	0.87	0.39	1.92