Diabetic glucolipotoxic cardiomyopathy: does it exist in humans?
Abstract

Type 2 diabetes has grown to pandemic proportions and associates with a high heart disease risk, particularly heart failure. Cardiac functional and structural changes in the absence of hypertension and coronary artery disease are termed diabetic cardiomyopathy (DCM). DCM is a multi-causal condition, however, diabetes-related metabolic derangements, including insulin resistance, leading to gluco-lipotoxicity are considered key factors in its development. A major limitation of these insights is that these are mere premises, primarily based on animal research. Recent progression in non-invasive imaging of the human heart has enabled the detailing of structural, functional and metabolic alterations in human DCM, challenging the common direct extrapolations from artificial animal models to humans. During the course of diabetes, quantitative and qualitative changes occur in cardiac function and metabolism, their interrelationship and the impact of co-morbidities and gender. These dynamic aspects have not received much attention, mainly due to the complexity of data acquisition in humans across the various stages of diabetes. Nevertheless, these time-dependent changes may have critical implications for the choice of treatment, especially regarding metabolic manipulation. Inasmuch as early metabolic intervention may favorably alter myocardial metabolic and functional remodeling, thus delaying progression to heart failure, it may also interfere with the early adaptive processes that aim to preserve cardiac function. Here, we critically review the available evidence in human DCM regarding cardiac metabolic abnormalities, their comparison to animal data and the relation between metabolism and function. Finally, we address the clinically relevant question whether and when DCM is amendable for metabolic treatment.

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General introduction to Diabetic Cardiomyopathy

Heart disease is the most common cause of death in type 2 diabetes (T2DM). Patients with T2DM are prone to develop coronary artery disease (CAD) and heart failure (HF). Relative to non-diabetic subjects, CAD risk may be as high as 3- to 4-fold in men up to 10- to 13 fold in women, relative to non-diabetic peers.(3;4) Particularly, T2DM increases the risk to develop HF or to die from HF to a greater extent in women as compared to men.(5;6) Cardiac structural and functional abnormalities exist already in asymptomatic T2DM patients, even in the absence of CAD or hypertension, due to diabetic cardiomyopathy (DCM).(7;8) Although DCM is a multi-factorial condition, diabetes-related metabolic derangements are often regarded as key contributors to the observed cardiac abnormalities.(8;9)

Most information with respect to the DCM phenotype and its underlying molecular mechanisms has emerged from studies in rodents that underwent genetic, pharmacological or dietary modifications (8;10-12). Although these data have certainly advanced our understanding of the potential mechanisms contributing to impaired myocardial function in DCM, still, the direct translation of these experimental findings to the human situation has proved invalid and therefore should be regarded with caution. Currently, the human DCM phenotype is thought to consist of left ventricular (LV) hypertrophy, LV diastolic dysfunction, myocardial insulin resistance, predominant reliance on fatty acid oxidation for myocardial energy metabolism and an inappropriate deposition of triglycerides (10-14). In particular the latter abnormalities, in the absence of reliable molecular proof from representative human cardiac tissue, are currently viewed as surrogates representing lipotoxicity. Indeed, lipotoxicity in animal models of DCM designates the deleterious consequences of lipid or fatty-acid overload in the myocardium leading to the formation of toxic intermediates, oxidative stress, mitochondrial dysfunction and ultimately apoptosis and cardiac dysfunction (10). All these adverse effects may be further aggravated by the concomitant occurrence of ischaemia, since the reliance on fatty acid oxidation as major energy source requires abundant oxygen (10). Thus, in the diabetic heart, abnormal substrate metabolism is presently regarded to be linked to impaired energy metabolism and consequently to dysfunction.

Based on these mainly preclinical data, it is advocated that improving the balance in cardiac substrate utilization, by enhancing insulin-mediated glucose uptake and lowering fatty acid supply, will ameliorate cardiac function and in the long-term, protect the heart from inappropriate remodeling and damage, particularly in response to cardiac stress. Indeed, to date, using metabolic manipulation, small-sized studies, including non-diabetic non-ischemic patients with idiopathic dilated cardiomyopathy (IDCM) treated with metoprolol,(15) carvedilol,(16) and trimetazidine,(17) showed improvement in ejection fraction and decreased myocardial NEFA utilisation,(16;17) without changing myocardial oxygen consumption.(15;17) In non-ischemic T2DM patients, a differential favourable effect of pioglitazone relative to metformin was found on LV compliance, function, cardiac work and substrate metabolism.(18) However, acute lowering of NEFA by acipimox in IDCM patients, with advanced LV functional impairment, rather deteriorated myocardial efficiency with no beneficial effect on function.(19) Also, in various clinical settings, metabolic manipulation has proven disappointing in diabetic subjects.(20-22) Thus, the Diabetes Mellitus Insulin-Glucose Infusion in Acute Myocardial Infarction II (DIGAMI II, n=1253) study,(21) and the CREATE-ECLA (n=20201) study (17.7% T2DM subjects),(22) failed to confirm the initial positive results from DIGAMI I with respect to a favorable prognosis after intensive metabolic care with insulin in diabetic patients in the acute phase of myocardial infarction (20). Interestingly, post hoc analyses revealed that the risk of non-fatal myocardial infarction and stroke was even increased significantly by insulin treatment.
while metformin was protective (23;24). Collectively, these findings have raised the awareness that the relationship of cardiac metabolism and function is extremely complex, and may likely differ according to the state of the disease.

Thus, in early human non-ischemic DCM this relationship of metabolism and function may be relatively weak as the heart seems to possess sufficient oxidative capacity to metabolize a wide array of substrates, including the excessively supplied fatty acids, due to adaptive changes.(25) However, the human phenotype of DCM changes over time, in parallel to and in interaction with the progression of diabetes. In the face of poorly controlled diabetes, concurrent insulin resistance, developing neurovascular complications, hypertension, inappropriate remodeling and particularly, with developing ischemia, the heart increasingly shows metabolic inflexibility.(26) Thus, the decreased ability of the compromised diabetic heart to readily switch between substrates may adversely affect its energy production, such that ultimately, even small changes in substrate supply will considerably aggravate the existing cardiac dysfunction.

In this review we critically address the structural and metabolic changes in human DCM and the current evidence regarding the link between cardiac metabolism and function and in particular, data supporting the existence of myocardial (gluco)lipotoxicity in human DCM. Since our current understanding of molecular pathways underlying the DCM phenotype is based on data obtained from animal studies, we may have to resource to these pre-clinical models but will clearly indicate how animal data will extend to humans. From the DCM phenotype is based on data obtained from animal studies, we may have to resource to these pre-clinical models but will clearly indicate how animal data will extend to human DCM.

Myocardial structure in human Diabetic Cardiomyopathy

Geometrical alterations

Left ventricular hypertrophy (LVH) has been regarded as a common hallmark of human DCM,(27) and is a typical finding rodent models of diabetes such as the ob/ob, db/db mice and ZDF rats.(14) However, more recent studies, using more advanced methodology, have refined these assumptions (7;25;28).

A sub-study (n=3220, = 7% diabetes) of the Framingham Heart Study showed that LVH was associated with a higher event rate, including death attributable to cardiovascular disease.(29) It was demonstrated that the relative risk for cardiovascular disease increased with each increment of 50 g m⁻² in LV mass (LVM) index by 49% in men and by 57% in women. From this same cohort it was shown that LVM was only significantly increased in women with diabetes, but not in diabetic men. (30) However, LVH was not found in subjects with IGM. In a later publication (n=2623) from the Framingham Heart Study, relations between LVM and glucose tolerance were assessed, showing a trend of increased LVM across categories of worsening glucose tolerance, that was highly significant in women, but only of borderline significance in men.(31) When additionally corrected for BMI, trend analyses lost significance in the normal glucose tolerance group. In a echocardiography substudy of the Hoorn Study (n=780), a significant association of increasing LVM with deteriorating glucose tolerance status was only observed in women, after adjusting for age, height, BMI, and mean arterial pressure.(32) A similar linear trend of LVM and glucose status was found for women and men alike in the diabetic subgroup (n=5201, = 52% diabetes) of the Cardiovascular Health
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Following correction for confounders in trend analyses, diabetes remained a predictor for LVM, albeit only of marginal significance (P=0.043). Evidence for increased LVM in diabetes was also obtained from the Strong Heart Study,(34-36) showing a progressive increase in LVM with the presence of diabetes or hypertension alone or the combination thereof, relative to those without either condition.(37) The above mentioned cohort studies all used echocardiography for the assessments of LVM. Echocardiography has been shown to be less accurate for the assessment of LVM, when compared with magnetic resonance imaging (MRI) due to the need for geometrical assumptions, subject characteristics (e.g. an unfavourable anatomy in obesity and gender specific entities causing a poor echo window) and its operator dependency.(38) Another point to take into account is that the above mentioned studies did not exclude (sub)clinical cardiovascular disease and no detailed information is available on (estimated) diabetes onset, which precludes conclusions regarding the pathophysiological role of diabetes per se in LVH. In the Multi-Ethnic Study of Atherosclerosis (MESA), another large cohort study (n=4491, 40% impaired glucose metabolism) of adults aged 45-84 without prior cardiovascular disease, MRI assessments showed increased LVM in white, black and Hispanic diabetes participants, but not in those with Asian background, which was more pronounced in women than in men.(39) However, after adjusting for covariates, differences in LVM disappeared in white, but remained in black and Hispanic diabetic participants. LVM was not different between participants with IGM and those with normoglycemia. The end-diastolic volume (EDV) was decreased in white and Hispanic men with impaired glucose metabolism and diabetes, but not in women. Following adjustments for classical covariates including gender, EDV was only decreased in whites and blacks with impaired glucose metabolism and diabetes. Information on EDV and LVM is relevant as a smaller EDV and increased LVM are indications for concentric remodeling. In a small-sized (n=24) MR study, LVM did not differ between patients with uncomplicated T2DM and matched controls, who had similar mean blood pressure and BMI.(7) Using MRI in 78 recently diagnosed Caucasian male T2DM patients, in whom inducible ischemia was excluded by dobutamine stress echocardiography, we did not find an increased LVM relative to age-matched controls.(25) However, after additional adjustment for blood pressure and BMI, the difference in LVM became significant between groups, implicating that blood pressure and BMI mediate the relation between LVM and diabetes (Rijzewik et al, unpublished data). In these T2DM versus age-matched control males, EDV was lower at similar estimates of filling pressure (E/E’) indicating decreased compliance and hence concentric remodeling in the early diabetic heart, which was in line with the MESA study.(40) In a study detailing changes in myocardial function and structure in patients with and without diabetes, who suffered from non-ischemic systolic versus diastolic HF, (41) LV stiffness was less a feature in systolic HF, however increased in diastolic HF and was most prominent in patients with diastolic HF and T2DM, paralleled by a shift from eccentric to concentric remodeling as evidenced by smaller LVMi and LVEDVi.(42) Another interesting finding repetitively emerging from the published evidence is that diastolic HF is more common in women than men.(43) Women referred for cardiac catheterization associated with HF symptoms, showed smaller LVEDV, however with similar LV end-diastolic pressures as men, indicating a female predominance for concentric remodeling.(44) The current geometrical picture is clearly a different picture of the diabetes heart as compared to the first clinical description by Rubler et al,(27) in which LV dilatation and systolic dysfunction were the prominent features. Indeed, from the above mentioned studies we may conclude that LVH in human DCM is not an unequivocal finding, especially not in the early and pre-diabetic stages, and that it shows gender and race specific variation. Importantly, the relation between LVH and diabetes may largely be driven by classical confounders, including hypertension, BMI and CAD. Myocardial stiffening and concentric
remodeling are (early) features of the non-ischemic T2DM human heart in both genders, but possibly with a female predominance. Finally, echocardiographic assessment to detail LV geometry has important drawbacks as compared to MRI.

**Left ventricular dysfunction in human diabetic cardiomyopathy**

As mentioned above, the initial publication of Rubler described DCM as an entity characterized by dilated cardiomyopathy with LV systolic dysfunction.\(^{(27)}\) Subsequently, LV diastolic filling abnormalities were recognized as earlier manifestations of diabetes-related cardiac dysfunction,\(^{(45,46)}\) which showed to be prognostic for all-cause and cardiac mortality.\(^{(47,48)}\) Studies evaluating LV function in different glucometabolic states have frequently used (tissue) Doppler echocardiography. Echocardiography is perfectly suited for the measurement of myocardial LV systolic and diastolic function, due to its high temporal and spatial resolution. Moreover, echocardiography is readily available and relatively cheap. Its disadvantages are its large inter-observer variability and poor window in obesity necessitating a higher number of participants in comparative studies for adequate powering. Alternatively, MRI is a valuable tool for the assessment of LV function, especially as it can be combined with precise measurements of LV geometry and the more experimental assessments such as MR spectroscopy to estimate various aspects of LV metabolism and contrast-enhancement to estimate scarring and fibrosis.\(^{(49)}\) Due to its accuracy and lower inter-observer variability, a higher power with less participant can be achieved. Cost and time consuming manual post-processing have traditionally been limitations, however automated LV function analyses have recently made these limitations less relevant.\(^{(50)}\)

Both echocardiography and MRI yield estimates of diastolic function by measuring transmitral flow velocities and chamber volume transients. In the early diastole (E) the LV relaxes and blood is drawn into the LV chamber. Early LV relaxation and filling are energy consuming processes, but may also be influenced by myocardial stiffness.\(^{(51)}\) Late diastolic filling (A) is due to atrial contraction, which account for 30% of LV chamber filling, and is mainly influenced by myocardial stiffening due to structural alterations.\(^{(42)}\) Besides the individual indices of E and A, the ratio of E/A is often used as a measure of diastolic function, with E/A ratio considered normal when between 0.75 and 2.0.\(^{(51)}\)

Diastolic LV filling abnormalities can hence be described by typical alterations in the transmitral flow pattern and their severity can be categorized such that an E/A ratio > 2.0 and < 0.75 denotes severe diastolic dysfunction.\(^{(52,53)}\) It needs to be emphasized that these indices are load dependent. In other words, the E/A ratio may be altered in the presence of increased filling pressure. However, pulsed tissue Doppler imaging enables non-invasive assessment of LV filling pressure by the E'/E ratio.\(^{(54)}\) Accordingly, combining E and E' from tissue MR imaging allows similarly adequate estimation of filling pressure by MRI and Doppler, both which are in good agreement with invasive measurements.\(^{(55)}\) Strain and strain rate (SR) analyses are more sensitive load independent indexes of LV diastolic and systolic function, which measure the amount and rate of myocardial deformation and therefore provide insight in the longitudinal, circumferential and radial mechanics of the heart.\(^{(56,57)}\)

Several factors have been identified as contributors to LV diastolic functional changes. In the Hoorn study, T2DM was independently associated with a 2.0-fold greater risk of LV systolic dysfunction and a 2.4-fold greater risk of LV diastolic dysfunction with hyperglycemia and hyperinsulinemia to gether explaining approximately 30% of the association of T2DM in LV systolic dysfunction and 40% of LV diastolic dysfunction.\(^{(58)}\) Other factors involved include impaired relaxation\(^{(59)}\), decreased LV distensibility and increased LV end-diastolic stiffness, \(^{(51,60,61)}\) and pericardial and right ven
tricular constraint (62;63) In addition diastolic blood pressure and heart rate are involved.(25)
Molecular studies in animal models of DCM, which mainly report LV systolic rather than diastolic dysfunction, have implicated myocardial steatosis, oxidative stress, mitochondrial dysfunction, altered calcium homeostasis and advanced glycation end products(AGEs) formation as contributors to LV dysfunction,(64) Non-invasive MR spectroscopy in human T2DM revealed myocardial triglyceride deposition,(65;66) oxidative stress,(67) and increased high-energy phosphate metabolism,(7) although not unequivocal,(25;68), as contributing factors to LV diastolic impairment in human T2DM. Since performing cardiac biopsies in uncomplicated human DCM is ethically unjustified, the only direct molecular evidence stems from more severe DCM phenotypes, usually compatible with advanced diabetes. Thus, biopsies obtained from T2DM patients with non-ischemic HF have found alterations in myosin heavy chain (MHC) expression,(69;70) and impaired calcium homeostasis as contributing factors (69;71). Finally, LV diastolic stiffness was shown to be caused due differential mechanisms in T2DM patients with diastolic HF (increased resting tension and cardiomyocyte hypertrophy) and systolic HF (myocardial AGEs deposition and fibrosis).
LV diastolic filling abnormalities can already be found in obese and insulin resistant individuals and in those with the metabolic syndrome.(7;72;73) In T2DM, even before structural cardiac abnormalities become manifest, 60% of patients without CAD or diabetes-related complications show LV filling abnormalities,(25;74;75) which is augmented during exercise.(76) Diastolic alterations in strain and strain rate were also shown in male patients with recently diagnosed, uncomplicated and well-controlled T2DM in whom inducible ischemia was ruled out.(66) LV filling abnormalities will continue to worsen with the progression of diabetes and the additional development of diabetes-related complications, including micro-angiopathy, autonomic neuropathy as well as comorbidities such as hypertension and coronary artery disease.(53)
Myocardial systolic function has traditionally been quantified by measuring LV ejection fraction (LVEF).(25) Patients with recent-onset diabetes have been reported to have a preserved systolic function, as reflected by a normal LVEF,(7;25) and hence systolic function has been regarded as a late phenomenon in DCM.(27) Compared to the LVEF, strain and strain rate (SR) analyses are more sensitive indices for LV systolic function. Several studies have demonstrated alterations in systolic strain and strain rate in T2DM patients with uncomplicated disease.(77-82) Accordingly, in addition to LV diastolic abnormalities, systolic alterations in strain and strain rate were observed in T2DM males with recently diagnosed diabetes in whom inducible ischemia was ruled out.(66) Changes in strain rate were also detected in isolated obesity, with obesity duration being the largest independent predictor of decreased strain rate.(83) Even in obese adolescents, relative to lean counterparts, strain reduction could be detected.(73)
LV filling dynamics may be gender specific. In the Framingham Study, Doppler indexes of LV diastolic function, were similar between men and women and gender was only a minor determinant.(84) However, in healthy middle-aged subjects (n=93), gender explained 32 to 57% of the variation in Doppler indexes of LV filling.(85) Moreover, in healthy subjects (n=67) more efficient early diastolic filling was shown in postmenopausal women than in age-matched men.(86) Furthermore, once HF develops, it seems that women are more prone to develop diastolic HF than men.(87) The aforementioned differences may partly be explained by differences in androgen and estrogen levels, of which the latter are associated with cardio-protection during the fertile age.(88) Following menopause however, the protective effects of estrogen disappear in women. Although risk factors are similar for men and women, relative contribution of these risk factors to HF risks may differ. For instance, hypertension prior to the development of HF is more common in women than in men,(89) and HF incidence is double in women aged 35-64 years compared to men.(90)
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Also, diabetes in one of the strongest predictors of HF in postmenopausal women with CAD. (91) In conclusion, even though it may take decades for LV functional changes to evolve into HF, from the above referred studies it becomes clear that alterations in myocardial LV diastolic and systolic function are early detectable findings in those with obesity, insulin resistance and T2DM, which can readily be detected by dedicated cardiac Doppler-ultrasound and MRI techniques. These early LV changes, although they are considered adaptive to the altered hemodynamic and metabolic situation, on the longer term, are no good-hearted bystanders in DCM, as they have been shown prognostic for all-cause and cardiac mortality. (47;48) Besides, LV dysfunction in T2DM patients has a high propensity to progress to HF, especially in women with T2DM. However, detailed and systematic data on gender differences at present are limited.

Rodent models versus human diabetic cardiomyopathy: relevant differences

Based on animal research, myocardial lipotoxicity is regarded as the resultant of successfully and progressively deregulated metabolic processes in the cardiomyocyte. It is assumed that obesity and insulin resistance, further aggravated by hyperglycemia, lead to increased NEFA fluxes overriding oxidative capacity, which in turn lead to the formation of toxic intermediates and triglyceride accumulation, uncoupling of oxidative metabolism with ROS formation and mitochondrial dysfunction resulting in energy depletion. (10;92) To date, most studies investigating myocardial metabolism have been performed in animal models in vivo or in rodent cardiomyocytes in vitro. Accordingly, research in rodent models of hyperglycemia and insulinopenia, often erroneously termed ‘type 1’ diabetes-models, as well as in obese, insulin resistant often genetically manipulated ‘type 2’ diabetes-models have put forward many candidate molecular mechanisms that could also apply to human DCM. (14) In particular studies in genetically manipulated leptin-signaling deficient ob/ob mouse, db/db mouse and the Zucker diabetic fatty (ZDF) rat have identified many interesting pathways leading to DCM, as the result of gluco-lipotoxicity, and has been welcomed and extrapolated as a major denominator of all abnormal findings in non-adipose organs in animals and T2DM humans alike. (8;10;92-94) However, whether gluco-lipotoxicity is a prominent feature in human T2DM and underlies human DCM at all stages remains arguable.

Rodent models progress from moderately obese, with concomitant hyperinsulinemia and glucose intolerance at an early age, to severe obesity and hyperglycemia in adulthood. Moreover, they show depressed systolic and diastolic LV function, increased LVM, increased myocardial lipid content and fatty acid oxidation and decreased cardiac efficiency. (14;95) The extent to which changes occur in obesity, plasma glucose, NEFA and triglycerides varies with the specific model. (95) However, leptin is a major bioactive anti-lipogenic hormone that can normalize ectopic lipid deposition in non-adipose tissues. (96) In spite of previous reports indicating some leptin resistance in obese insulin resistant individuals and those with T2DM, (97;98) it is highly unlikely that the extreme phenotype resulting from leptin deficiency in the rodent model is applicable to the human situation and that the underlying phenotypic and molecular abnormalities in the hearts of these models fully represent the mechanisms contributing to human DCM. (99;100)

Although many similarities seem to exist between animal models of DCM and human DCM, direct translation of data from animal models should be done with caution. (101) Differences in the length of the cardiac cycle, genetic heterogeneity, as well as differences in lifestyle among humans, contrast with the controlled situation of animal experiments (specific chow supply, homogeneity of inbred rodent species etc). Another marked difference is that rodents hardly develop atherosclerosis since they typically have very low levels total and LDL cholesterol and high HDL cholesterol levels, which...
renders them very suitable for research in non-ischemic DCM.(14;102) Also, hypertension is not a prominent feature for example in ZDF rats.(14) However, in human obesity and diabetes, CAD, microvascular disease and hypertension are important additional stressors compromising myocardial metabolism and performance as diabetes progresses.(103) Besides, an important, often neglected factor in the development of the DCM phenotype is the interaction of the metabolic disturbances and time course: in rodents, the myocardium is exposed to extreme metabolic abnormalities that are mostly short-lived whereas in humans relatively mild but chronic alterations affect the heart. Moreover, implementation of early treatment to delay complications alters the natural evolution of DCM in humans, thus hampering the detection of potentially contributing factors. For instance, guidelines for the treatment of T2DM, recommend to start cholesterol-lowering therapy,(104) irrespective of the actual lipid levels, and to treat blood pressure aggressively to low-normal levels. (104) Frequently used drugs include statins and inhibitors of the renin-angiotensin-aldosterone system (RAAS), which may subsequently blunt the established association between LV changes, dyslipidemia and blood pressure, not only by their lipid- and blood pressure lowering effects, but also through their respective pleiotropic effects, including the inhibition of apoptosis and fibrosis and cardiac remodeling.(105;106) Thus, establishing the human phenotype of DCM, its determinants, the underlying mechanisms and the relevant interrelationships may be complicated and likely to change during the course of diabetes. As a consequence, it may be expected that the human situation will further divert from the frequently used animal models over time.

**Metabolism in human diabetic cardiomyopathy**

**Myocardial perfusion in diabetic cardiomyopathy**

Insulin resistance, in addition to beta-cell dysfunction, is an important hallmark of human T2DM. Insulin resistance contributes to the typical T2DM-related dyslipidemia, which in combination with hyperglycemia, the ensuing pro-inflammatory state and increased oxidative stress, accelerate the development of coronary artery disease (CAD) in these patients.(107;108) T2DM patients with early onset diabetes (< 60 years of age) as compared to age-matched non-DM populations, have an increased risk of major CAD events and mortality which appears to be a CAD equivalent.(109) The T2DM estimated 4-year survival rate of T2DM patients following a first acute MI is only 50%. (110) The poorer prognosis of acute myocardial infarction in diabetic patients, however, appears not to be explained by a larger infarct size but probably by adverse effects of the diabetic state itself on myocardial function.(111)

Endothelial dysfunction, referred to as an impaired ability of the endothelium to properly maintain vascular homeostasis, is an early event in diabetic macrovascular disease and precedes the development of morphologic vascular damage, as detectable by non-invasive ultrasound examination. (112;113) Thus, endothelial dysfunction, should rather be termed endothelial “dysfunctions” as it comprises disturbances of vasodilatory, anti-thrombogenic, anti-inflammatory and anti-proliferative capacities.(114) Endothelial functions can be estimated by ultrasound of the brachial artery by flow-mediated dilation (FMD), which was shown to be decreased in T2DM patients.(115) Moreover, insulin-mediated skeletal muscle blood flow was shown to be impaired in skeletal muscle of T2DM patients.(116)

These actions can also be extended to the heart as hyperinsulinemia increases myocardial perfusion in healthy humans.(117) T2DM patient are prone to develop microvascular disease, which is considered to be one of the contributing factors to the development of DCM.(118) In the unstressed non-ischemic T2DM human heart, resting perfusion is not altered as compared to healthy individuals.(25) However, in those patients with more accentuated insulin resistance and an un-
favorable metabolic pattern associated with hepatic steatosis, myocardial resting perfusion is decreased.\(^{119}\) To emphasize, coronary flow reserve in T2DM with CAD was found to be 28% lower in patients with hepatic steatosis relative to low liver fat subjects with a similar degree of CAD, indicating more severe coronary dysfunction.\(^{120}\) Insulin resistance per se was associated with reduced coronary vasoreactivity in healthy humans,\(^{121}\) and decreased coronary flow reserve in non-ischemic T2DM.\(^{122}\) These effects are striking, as coronary vasoreactivity in T2DM patients is impaired to the same extent as in patients with CAD.\(^{123}\)

The endothelium plays a key role in the regulation of arterial tone and blood flow by regulating production of both vasodilator molecules, such as nitric oxide (NO) and prostacyclin, and vasoconstrictor molecules, including endothelin 1 and angiotensin II.\(^{124}\) Mechanisms by which T2DM is believed to induce endothelial dysfunction include altered cell signaling, i.e. insulin signaling pathways associated with insulin mediated activation of endothelial NO synthase (eNOS) are impaired, increased oxidative stress, pro-inflammatory activation of the endothelium, activation of protein kinase C, mitochondrial dysfunction and increased endothelial fatty-acid oxidation.\(^{124;125}\) Although the increased susceptibility to HF is evident,\(^{5}\) the underlying mechanisms remain poorly understood but altered perfusion may be contributory. Lean young women are more insulin sensitive than equally fit men related to enhanced muscle but not heart insulin sensitivity, which is similar.\(^{126}\) In young, healthy, non-obese, premenopausal, sedentary women, myocardial perfusion was not different from age and BMI matched males.\(^{127}\) However, in obese women with high insulin levels, relative to lean and obese men, myocardial perfusion was increased and female gender independently predicted myocardial perfusion changes, accounting for 22% of the variability.\(^{128}\) Based on epidemiological studies, premenopausal women are relatively protected against CAD, as compared to men, which may be related to differences in sex hormones as previously mentioned.\(^{88}\) When menopausal, however, these benefits fade out. In women, microvascular dysfunction is more pronounced than in men,\(^{129}\) and among STEMI (ST segment Elevation Myocardial Infarction) patients, 10%–25% of women have normal angiograms, compared to 6%–10% of men, which was explained by a higher prevalence of microvascular abnormalities in women compared to men, that had resulted in STEMI.\(^{130}\) The above-mentioned studies provide evidence for a regulatory effect of insulin on myocardial micro- and macro-vasculature and indicate that insulin resistance influence both negatively.\(^{117;119;123}\) These effects can already be found in obese subjects and T2DM patients without coronary artery disease, being therefore an early finding in the DCM phenotype.

**Myocardial metabolism in diabetic cardiomyopathy**

Extensive human cardiac metabolic research has traditionally been difficult, due to the invasive nature of catheter studies, but also high cost, technical challenges, radiation exposure and related ethical considerations in PET and SPECT studies. In vivo investigations of human myocardial metabolism dates back to the early decades of the 19th century.\(^{131;132}\) Based on catheterisation studies in the early 1950’s, Bing et al acknowledged that the human heart was able to handle all major nutrients, i.e. glucose, fatty acids, ketone bodies and amino acid.\(^{133}\) They also appreciated alterations in myocardial metabolism in diabetes and concluded that the diabetic heart is deficient in the metabolism of carbohydrates, proteins and fatty acids.\(^{134}\) However, these early studies were additionally hampered by the fact that they, included very heterogeneous diabetic populations, in terms of gender, diabetes duration (0.5 – 18 years), age (21-57 years), treatment (many on insulin, but also diet alone) and type of diabetes, i.e. both T1DM and T2DM.\(^{134}\) More recent studies have used advanced imaging (nuclear) techniques such as single photon emission computed tomography (SPECT), positron emission tomography (PET) and magnetic resonance spectroscopy (MRS) to study several aspects of myocardial metabolism. The development of dedicated tracers have made it possible to specifically track and quantify (regional) myocardial perfusion, glucose, fatty-acid,
lactate metabolism and oxygen consumption. Data from PET studies can be analyzed using compartment modeling, which accurately describe physiological pathways in a mathematical way. Figure 1 shows cardiomyocyte metabolism, including dedicated tracers that can be used for non-invasive quantification of cellular metabolic processes.

Cardiac substrate uptake, including non-esterified fatty acids (NEFA), glucose and lactate, is largely receptor mediated, however NEFA may enter the cell by diffusion. Following uptake, NEFA are converted to fatty acyl-CoAs, which are then transported into the mitochondria through carnitine palmitoyltransferase (CPT) 1 and 2. There fatty acyl-CoAs undergo β-oxidation (β-ox) generating acetyl-CoA and the reducing equivalents nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH). Anaerobic degradation of glucose can also lead to generation of lactate. In the presence of oxygen, pyruvate is transported into the mitochondria through the multienzyme complex pyruvate dehydrogenase (PDH). Pyruvate is converted to acetyl-CoA, with the formation of NADH, and fatty acyl-CoA are converted to acetyl-CoA with formation of NADH and FADH. Oxidation of acetyl-CoAs in the citric acid or tricarboxylic acid (TCA) cycle generates CO₂ and guanosine triphosphate (GTP) as well as NADH and FADH. Electrons (e⁻) derived from NADH/FADH, are transferred via electron transport complexes I to IV from the electron transport chain (ETC). Here, electrons are transferred to oxygen which is then reduced to water and consequently a proton (H⁺) gradient is formed. As protons re-enter the mitochondria through ATP-synthase, adenosine triphosphate (ATP) is generated from adenosine-diphosphate (ADP). Cardiac substrate and oxidative metabolism in humans can be assessed non-invasively by positron emission tomography (PET) or single-photon emission computed tomography (SPECT) using dedicated tracers (displayed in rectangles below their natural substrates). Cardiac molecular imaging is used to assess several metabolic process (displayed in ovals): myocardial triglyceride content (¹H-MRS), mitochondrial high-energy metabolism (³¹P-MRS), and pyruvate metabolism (¹³C-MRS). For description tracers see text.
Glucose and fatty-acids are the hearts main fuels.(137) Glucose is transported into the myocyte by the GLUT4 receptor and subsequently phosphorylated to glucose-6-phosphate. In the process its metabolite is brought into the mitochondrion and is subsequently oxidized in the Krebs cycle (Figure 1). PET studies of myocardial glucose metabolism in human diabetes have used fluorodeoxyglucose (18F-FDG), but the use of 13C-glucose has been documented as well.(138) The main difference between these tracers is that 18F-FDG is a glucose analogue, which undergoes only the first step in metabolism i.e. conversion to glucose-6-phosphate, after which it is trapped. In contrast, 13C-glucose is fully metabolized and thus also provides information about glucose oxidation. Consequently, different mathematical models are developed to model tracer kinetics and allow estimations of myocardial glucose metabolism in health and disease.(139;140) Table 1 summarizes cardiac PET studies in human insulin resistant states, including obesity, impaired glucose metabolism and T2DM. Most of these studies have been performed under euglycemic hyperinsulinemic conditions for the obvious reason that without insulin stimulation virtually no glucose-tracer will be taken up by the insulin resistant heart in the fasting state, moreover intra and inter individual variability in glucose levels in the fasting and post-prandial state importantly influence glucose uptake and complicate post-assessment statistics.

Although animal studies have convincingly demonstrated a reduction in myocardial glucose metabolism in insulin resistant and hyperglycemic animal models, (93;141;142) early small-sized PET studies in humans were less conclusive (Table 1). However, subsequent human all showed reduced insulin-stimulated glucose uptake in insulin resistant states without and with coronary artery disease (in unaffected regions), as compared to controls. (25;143;144) Recently, it was shown that a decrease in insulin-stimulated myocardial 18F-FDG uptake in T2DM patients was paralleled by a reduction in sarcolemmal GLUT4 transporters, as determined in endomyocardial biopsies.(144) Thus, altered glucose metabolism, in particular impaired insulin-stimulated glucose uptake is an important characteristic of the human DCM phenotype, and decreased sarcolemmal GLUT4 may contribute to the defect.

Myocardial fatty-acid metabolism has been widely studied in diabetic animal models.(95) It is generally assumed that in the normal heart, fatty-acid oxidation accounts for 60-70% of cardiac energy production.(137;145) The myocardium uses two sources of fatty-acids, including albumin bound fatty-acids derived from adipose tissue lipolysis and fatty-acids from hydrolysis of triglyceride rich lipoproteins by the enzyme lipoprotein lipase, which is located on the surface of endothelial cells. (95) Fatty-acids are taken up into the myocyte by active transport via several proteins (including fatty-acid translocase/CD36, fatty-acid binding protein (FABPpm) and fatty-acid binding proteins (FATP)) and by simple diffusion.(146) Following uptake, fatty-acids are transformed to fatty acyl-CoA by Acetyl-CoA synthetase and bound to intracellular transport proteins. These are then esterified or metabolized into the Krebs cycle (Figure 1). Depending on the questions asked, fatty-acid metabolism can be measured in the fasting post-prandial or clamp condition. Several tracers are available (Figure 1). The 18F-FTHA and 113I-heptadecanoic acid are trapped in the β-oxidation, whereas 14C tracers are fully metabolized, thereby providing besides fatty-acid uptake insight into fatty-acid esterification and oxidation. Most studies in animal models of T2DM have shown increased myocardial fatty-acid uptake and oxidation.(92;93;142;147-151) Few non-invasive human PET and SPECT studies have been performed in insulin resistant states, including obesity and impaired glucose tolerance (Table 1). Most of these studies included a limited number of participants, used different tracers with inherent different modalities, and applied various approaches to data analyses. As a consequence, these studies are far from conclusive with respect to fatty-acid metabolism in the insulin resistant heart. Of interest, fasting plasma fatty-acid levels are frequently increased in animal models,(95) but this is generally not the case in T2DM (in contrast to postprandial fatty-acid levels and fatty-acid levels under hyperinsulinemia).(25;66;119;143;145)
Table 1. Non-invasive assessment methods in human diabetic cardiomyopathy

<table>
<thead>
<tr>
<th>Author/reference</th>
<th>Population</th>
<th>Method</th>
<th>Tracer</th>
<th>Findings (patients versus controls/treatment)</th>
<th>function / structure and correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maki M, et al (232)</td>
<td>T2DM, CAD</td>
<td>PET</td>
<td>8F-FDG</td>
<td>↓ MGU</td>
<td>None</td>
</tr>
<tr>
<td>Uutinen T, et al (233)</td>
<td>T2DM</td>
<td>PET</td>
<td>8F-FDG</td>
<td>↓ MGU</td>
<td>↑ LVM</td>
</tr>
<tr>
<td>Sandegaard H, et al (234)</td>
<td>T2DM, T2DM+CAD</td>
<td>PET</td>
<td>8F-FDG</td>
<td>↓ MGU</td>
<td>None</td>
</tr>
<tr>
<td>Nuutila P, et al (235)</td>
<td>T1DM</td>
<td>PET</td>
<td>8F-FDG</td>
<td>↓ MGU</td>
<td>None</td>
</tr>
<tr>
<td>Peterson L, et al (167)</td>
<td>NGT, NGT obese</td>
<td>PET</td>
<td>13C-glucose</td>
<td>↓ MGU</td>
<td>↑ LVM, ↑ CO</td>
</tr>
<tr>
<td>Ohlaker T, et al (236)</td>
<td>T2DM</td>
<td>PET</td>
<td>8F-FDG</td>
<td>↓ MGU</td>
<td>None</td>
</tr>
<tr>
<td>Ylikoski L, et al (237)</td>
<td>T2DM, T2DM+Hyper tension</td>
<td>PET</td>
<td>8F-FDG</td>
<td>↓ MGU (T2DM without hypertension)</td>
<td>None</td>
</tr>
<tr>
<td>Paternoster L, et al (239)</td>
<td>T2DM+CAD</td>
<td>PET</td>
<td>8F-FDG</td>
<td>↓ MGU</td>
<td>None</td>
</tr>
<tr>
<td>Voipio-Pulkki L, et al (240)</td>
<td>T2DM+CAD</td>
<td>PET</td>
<td>8F-FDG</td>
<td>↓ MGU</td>
<td>None</td>
</tr>
<tr>
<td>Itoz P, et al (143)</td>
<td>T2DM, T2DM+CAD, T1DM</td>
<td>PET</td>
<td>8F-FDG</td>
<td>↓ MGU (T2DM+CAD)</td>
<td>MGU ↔ EF</td>
</tr>
<tr>
<td>Herrera P, et al (241)</td>
<td>T1DM</td>
<td>PET</td>
<td>13C-glucose</td>
<td>↓ MGU</td>
<td>None</td>
</tr>
<tr>
<td>Rijzewijk L, et al (25)</td>
<td>T2DM, NGT</td>
<td>PET</td>
<td>8F-FDG</td>
<td>↓ MGU (T2DM)</td>
<td>No correlation between DF and glucose metabolism, LVM =</td>
</tr>
<tr>
<td>Cook S, et al (144)</td>
<td>T2DM, LVD</td>
<td>PET</td>
<td>8F-FDG</td>
<td>↓ MGU, ↑ IRS1-Pi3K activity, GLUT 4 sarcolemmal expression and in vesicles, GLUT 4 vesicle dysfunction</td>
<td>T2DM all EF &gt; 40%</td>
</tr>
<tr>
<td>Van der Meer, et al (18)</td>
<td>T2DM PIO vs metformin</td>
<td>PET</td>
<td>8F-FDG</td>
<td>Pio ↑ MGU, Metf ↓ MGU</td>
<td>No correlation in Δ DF and glucose metabolism, Pio ↑ E dec Peak and EDVI with similar E/F, i.e. improved compliance</td>
</tr>
<tr>
<td>Lautamäki (242)</td>
<td>T2DM+CAD Rosi vs placebo</td>
<td>PET</td>
<td>8F-FDG</td>
<td>Ros ↑ MGU</td>
<td>Rosi ↓ EF</td>
</tr>
<tr>
<td>Hällsten (243)</td>
<td>T2DM Rosi vs Metformin</td>
<td>PET</td>
<td>8F-FDG</td>
<td>Ros ↑ MGU, Metf = MGU</td>
<td>None</td>
</tr>
<tr>
<td>Rijzewijk L, et al (119)</td>
<td>T2DM-FL+, T2DM-FL-</td>
<td>PET</td>
<td>8F-FDG</td>
<td>↓ MGU (T2DM-FL+)</td>
<td>No correlation between DF and glucose metabolism, LVM =</td>
</tr>
<tr>
<td>Lautamäki (120)</td>
<td>T2DM-FL+, T2DM-FL-</td>
<td>PET</td>
<td>8F-FDG</td>
<td>↓ MGU (T2DM-FL+)</td>
<td>None</td>
</tr>
</tbody>
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Table continues on next page
## Findings

<table>
<thead>
<tr>
<th>Author/reference</th>
<th>Population</th>
<th>Method</th>
<th>Tracer</th>
<th>Myocardial NEFA metabolism</th>
<th>function / structure and correlations</th>
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</thead>
<tbody>
<tr>
<td>Turepine A et al (244)</td>
<td>IGT</td>
<td>PET</td>
<td>$^{13}$F-FTHA</td>
<td>= MFAU</td>
<td>None</td>
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<tr>
<td>Knuuti J et al (245)</td>
<td>IGT</td>
<td>PET</td>
<td>$^{13}$C-palmitate, $^{13}$C-acetate</td>
<td>= MFAU / = β-oxidation</td>
<td>None</td>
</tr>
<tr>
<td>Kuikka J et al (246)</td>
<td>T2DM</td>
<td>SPET</td>
<td>$^{13}$H-hDA</td>
<td>= MFAU / ↑ elimination</td>
<td>Rest EF, ↓ stress EF</td>
</tr>
<tr>
<td>Turepine A et al (247)</td>
<td>T2DM, T1DM, IGT</td>
<td>SPET</td>
<td>$^{13}$H-hDA</td>
<td>↓ MFAU / β-oxidation in IGT</td>
<td>↑ Posterior wall and septum ↓ LVMI in IGT</td>
</tr>
<tr>
<td>Herrera P et al (241)</td>
<td>T1DM</td>
<td>PET</td>
<td>$^{13}$C-palmitate, $^{13}$C-acetate</td>
<td>↑ MFAU / ↑ MFAO / ↓ MVO₂</td>
<td>None</td>
</tr>
<tr>
<td>Rijzewijk L et al (25)</td>
<td>T2DM, NGT</td>
<td>PET</td>
<td>$^{13}$C-palmitate</td>
<td>↑ MFAU / ↑ MFAO / MFAE</td>
<td>No correlation between DF and palmitate metabolism, LVM =</td>
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<tr>
<td>Rijzewijk L et al (119)</td>
<td>T2DM-FL+, T2DM-FL-</td>
<td>PET</td>
<td>$^{13}$C-palmitate</td>
<td>= MFAU / = MIAO / = MFAE</td>
<td>No correlation between DF and palmitate metabolism, LVM =</td>
</tr>
<tr>
<td>Van der Meer et al</td>
<td>T2DM PIO vs metformin</td>
<td>PET</td>
<td>$^{13}$C-palmitate</td>
<td>Pio = MFAU / = MFAO / = MFAE ↑</td>
<td>No correlation in Δ DF and palmitate metabolism, Pio ↑ E dec Peak and E DVI with similar E/E', i.e. improved compliance</td>
</tr>
<tr>
<td>Peterson L et al (167)</td>
<td>NGT, NGT obese</td>
<td>PET</td>
<td>$^{13}$C-palmitate</td>
<td>= MFAU / = MIAO / = MVO₂</td>
<td>↑ LVM, ↑ CO</td>
</tr>
</tbody>
</table>

## Myocardial high-energy phosphate metabolism

<table>
<thead>
<tr>
<th>Author/reference</th>
<th>Population</th>
<th>Method</th>
<th>Tracer</th>
<th>Findings (patients versus controls/treatment)</th>
<th>function / structure</th>
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<td>Diamant M et al (7)</td>
<td>T2DM</td>
<td>$^{31}$P-MRS</td>
<td>-</td>
<td>= PCr/ATP</td>
<td>↓ DF, DF ↔ PCr/ATP</td>
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<tr>
<td>Scheueriann-Freestone M, et al (68)</td>
<td>T2DM</td>
<td>$^{31}$P-MRS</td>
<td>-</td>
<td>= PCr/ATP</td>
<td>= DF</td>
</tr>
<tr>
<td>Metzler B, et al (248)</td>
<td>T1DM</td>
<td>$^{31}$P-MRS</td>
<td>-</td>
<td>= PCr/ATP</td>
<td></td>
</tr>
<tr>
<td>Rijzewijk et al, (25)</td>
<td>T2DM, NGT</td>
<td>$^{31}$P-MRS</td>
<td>-</td>
<td>= PCr/ATP</td>
<td>No correlation between DF and PCr/ATP, LVM =</td>
</tr>
</tbody>
</table>

T2DM/T2DM, type 1/2 diabetes mellitus; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; CAD, coronary artery disease; PET, positron emission tomography; SPET, single photon emission tomography; $^{31}$P-MRS, phosphorus magnetic resonance spectroscopy; MGU, myocardial glucose uptake; NEFA, non-esterified fatty acids; MFAU, myocardial fatty acid uptake; MFAO, myocardial fatty acid oxidation; LVO₂, myocardial oxygen consumption; PC/ATP, phosphocreatine-to-adenosine-triphosphate ratio; DF, diastolic function; LVD, left ventricular dysfunction; LVM(I), left ventricular mass (index); EF, ejection fraction; CO, cardiac output; MTO, myocardial triglyceride content; EFR, early peak flow rate; E dec Peak, E deceleration Peak; E/E' = estimate of LV filling pressure; T2DM-FL+/- = T2DM patient with fatty liver/ non fatty liver. ↑, increased; ↓, decreased; =, no difference; ↔, no correlation.
Recently, a large-sized PET study, specifically designed to evaluate myocardial metabolism in the non-ischemic human T2DM heart was performed, and showed increased 11C-palmitate uptake and oxidation, supporting the observations from animal studies.(25) In patients with idiopathic dilated cardiomyopathy fatty-acid metabolism has been shown to be decreased.(152) Interestingly, with worsening of EF as well as insulin sensitivity (defined as HOMA < 1.87), myocardial fatty-acid uptake and oxidation were gradually upregulated. This finding seems contrary to what may be expected, as it suggest that the metabolic shift is modulated and counteracted by 1) further deterioration of LV function and 2) development of insulin resistance. In other words LV function itself modulates metabolism and insulin resistance further promotes the shift toward myocardial NEFA, at the cost of glucose metabolism.(152) These metabolic alterations result in the deterioration of energy efficiency of the heart in severe dilated CMP, as a greater reliance on fatty-acid metabolism is related to increased oxygen consumption.

Thus, although evidence from human studies is limited, increased fatty-acid metabolism seems to be part of the early DCM phenotype and impaired LV function in non-diabetic patients with HF reciprocally increases NEFA metabolism which is further aggravated by concomitant insulin resistance. It has been suggested that alterations in myocardial metabolism may underlie the gender-specific differences in prevalence and manifestation of a variety of cardiac disorders,(153) however, limited data are available. Myocardial oxygen consumption, glucose extraction and utilization, but not fatty-acid metabolism, was shown to be increased in young, sedentary, normal weight healthy women as compared to men, and to be independently related to the female gender (n=25). (127) In a larger study investigating gender-related impact of obesity on cardiac metabolism (n=86), female gender independently predicted higher cardiac work, lower efficiency, lower glucose utilization and higher fatty acid utilization and oxidation, suggesting that gender-related differences in myocardial metabolism may ultimately affect the development of, and adaptation to, obesity-related cardiac disease. (128) Currently, no studies have specifically phenotyped differences in myocardial metabolism between race, however in the former study no racial differences in any of the outcomes were observed.(128)

The use of animal models have generated important hypothesis concerning causes underlying DCM. The various differences between rodents and men, however, should be acknowledged. The human DCM phenotype includes a shift from glucose to fatty-acid metabolism. Although data is limited, gender related myocardial metabolic differences may influence myocardial metabolism and possibly explain gender differences in obesity related cardiac disease.

Myocardial triglyceride accumulation: data from 1H-MRS

Increased myocardial fatty-acid metabolism in fore-mentioned animal models of obesity and insulin resistance have shown myocardial lipid accumulation, which was paralleled by cellular apoptosis, metabolic and contractile dysfunction and have previously been reviewed extensively elsewhere.(10;154) Moreover, in ZDF rats impaired myocardial glucose metabolism, but not fatty-acid metabolism (both measured using 18F-FDG and 13C palmitate PET), was associated to LV diastolic (E deceleration time) and systolic functional (fractional shortening) changes.(93) Studies evaluating steatosis in the human heart have mainly relied on proton magnetic resonance spectroscopy (1H-MRS), although some endomyocardial biopsy studies to quantify lipid content have been performed. Compared to skeletal muscle, 1H-MRS imaging of the heart has been challenging, due to the constant movement of the heart and the presence of adjacent pericardial fat, which may interfere with the signal.(49) To avoid contamination of the assessment with pericardial fat, spectroscopy data are therefore acquired in the interventricular septum region in combination with respiratory motion correction.(155)
A limited number of human studies have addressed the association of cardiac steatosis and function in humans. In obese patients with IGT and T2DM, relative to lean and younger controls, an increase in myocardial triglyceride content was found, but no relation was found with diastolic or systolic functional measurements. In another study, an elevated myocardial triglyceride content was found in clinically healthy subjects across a wide range of BMI, which was accompanied by increased LV mass and a subtle reduction of septal wall thickening, a measure of regional systolic function. However, LV ejection fraction was unrelated to myocardial triglyceride content. In males in well-controlled T2DM relative to age- and BMI-matched controls an independent association of decreased LV diastolic functional parameters, but not with systolic parameters, and myocardial triglyceride content, as measured by MRI and ^1H-MRS was found. In another study evaluating male T2DM patients without evidence of ischemic heart disease, who were divided in a high and low myocardial triglyceride group as determined by ^1H-MRS, greater impairments of biventricular myocardial strain and SR, but similar biventricular volumes and EF compared to patients with low levels of myocardial triglyceride were found. Moreover, myocardial triglyceride accumulation was an independent correlate of biventricular myocardial strain and SR in these patients. Sharma et al., performed myocardial biopsies in n=27 (10 with T2DM) patients (M/F 18/9) with NYHA class IV non-ischemic HF as well as ZDF rats to quantify intramyocardial lipids. It was hypothesized that intramyocardial lipid accumulation is a common feature of non-ischemic heart failure and associated with similar changes as in ZDF rats. Eight out of 27 HF subjects showed a moderate increase in lipid deposition. T2DM HF patients had increased (P<0.05) intramyocardial lipid depositions as compared to lean non-diabetic subjects without heart failure, which was around 4 times as high. However no significant difference was found in obese HF patients without diabetes. In comparison, using ^1H-MRS, Rijzewijk et al., found only a 1.3 time increase in myocardial triglyceride content in non-ischemic hearts of patients with well-controlled uncomplicated overweight T2DM (n=78, all Caucasian males), who had mere subtle LV diastolic functional impairments. Because all human subjects in the study by Sharma et al., had severe end-stage HF, LV contractile function was similarly severely impaired in all patients hence not allowing to detect a meaningful relationship of lipid accumulation and function. In the same study, the ZDF rats had a 50-fold increase in myocardial lipids as compared to ZL rats, which was paralleled by decreased cardiac power. Taking together the clinical and preclinical data, the spectrum of DCM seems to comprise at the one end patients with uncomplicated T2DM who have only mildly elevated myocardial triglycerides, as compared to matched non-diabetic controls,(65,158) which is up to 3-4-fold increased in obese patients with end-stage HF and T2DM. At the far end of the spectrum the leptin-deficient ZDF rat model exhibits a massive increase in myocardial lipid content, with concomitant functional loss, however not to the extent as might be expected when compared to the human non-DM failing hearts.

Is gluco-lipotoxicity a key feature of human diabetic cardiomyopathy?

Based on the above-mentioned studies, the question arises whether the changes in substrate fluxes and myocardial triglyceride levels in human DCM cause activation of the pathways that ultimately result in lipotoxicity as observed in the more severe rodent models of DCM (25,70,93). It has to be kept in mind that alterations in substrate metabolism,(159) and increased myocardial triglyceride content are features of the ‘normally’ aging heart. Accordingly, cardiac TG content was related to age, independently of BMI.(160) In addition, stressors like hypertension directly impact on high-energy phosphate metabolism in humans.(161) Moreover, the rodent model is short lived and the consequences of lipotoxicity are already significant at 10-14 weeks of age. It seems that in
These rodent models the abnormalities are more severe and occur at an accelerated pace, within a short time-frame in contrast to the chronic, initially (metabolically) more mild course of human obesity-related insulin resistance and T2DM, in which the cellular consequences require years before causing clinically manifest and measurable abnormalities, leave alone severe LV dysfunction, microvascular disease and ischemia. The initially increased myocardial lipid metabolism in early human T2DM, may therefore not be equivalent to the lipotoxicity as detailed in rodent models. In view of this, however, it is not unlikely that as diabetes and concomitant cardiac and vascular disease progress in humans, that the biochemical and molecular changes compatible to lipotoxicity can no longer be compensated for, and that through increasing lipotoxicity-related cellular damage, metabolic changes become more closely linked to LV dysfunction.(65,158) Another relevant question is whether ectopic lipid/triglyceride accumulation in the heart has the same consequence for the cardiologist as the fatty liver for the hepatologist? Contrary to the heart, hepatic steatosis is undisputed and very common in human T2DM, leading to non-alcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH),(162) accompanied by clear associations between steatosis, perfusion and metabolism.(163)

The link between metabolism and function, does it only exist in the compromised human heart? The direct relation between systemic and myocardial metabolism and function has not been firmly established in human DCM, since most studies addressing cardiac metabolism did not measure functional variables and vice versa. Myocardial uptake of various substrates is dependent on the arterial concentration. Consequently the contribution of any one substrate leads to a decreased contribution by the others due to competition for the available oxygen, which sets the rate of mitochondrial oxidative metabolism.(145) Glucose is a more efficient fuel as less oxygen is needed to oxidize 1 mole of glucose relative to NEFA.(145) The uncompromised human heart can easily adapt to its needs by increasing uptake and oxidation of all major substrates.(164,165) Moreover, the uncompromised heart is able to readily switch between substrates, as dictated by the circumstances (i.e. fasting and feeding state), e.g. to switch to the most energy efficient substrate, in order to maintain ATP production. The ability to switch between substrates was referred to as metabolic flexibility of the heart by Heinrich Taegtmeyer, in analogy with David Kelley’s findings in skeletal muscle.(26,166) Metabolic flexibility is compromised when insulin resistance develops, as this leads to impaired insulin-mediated glucose uptake and, in a reciprocal manner, to increased fatty-acid uptake in the non-ischemic human T2DM myocardium.(25) In animal models of DCM, the upregulation of fatty-acid metabolism was shown to be mediated by PPARα activation by increased levels of (long-chain) fatty-acids.(26) A greater reliance on fatty-acid, relative to glucose metabolism for ATP production decreases myocardial energy efficiency in obese women.(167) In a group of T2DM patients a correlation was shown between myocardial glucose uptake and the ratio of phosphocreatinine to adenosine-tri-phosphatase (PCr/ATP).(119) Also, the PCr/ATP ratio was shown to be reduced in T2DM patients.(7,68) However, this could not be confirmed in the largest study hitherto performed to address this subject, which was the only study actively excluding inducible ischemia by dobutamine-stress echocardiography.(25) Moreover, only one of these studies showed a relation to LV diastolic function.(7) Thus in the non-compromised T2DM heart altered substrate metabolism does not necessarily translate into decreased high-energy phosphate metabolism, which seems disease-stage dependent as PCr/ATP is obviously decreased in HF.(25,168) Therefore, the initial impact on energy generation in the well oxygenated heart is, at best, limited and not detectable with the current methods.

Treatment of these non-ischemic T2DM patients for 24 weeks with the PPARγ agonist pioglitazone improved insulin resistance and increased myocardial insulin-mediated glucose uptake parallel to a modest diastolic LV improvement, which where however not directly related.(18) No changes were found in the ratio of PCr/ATP or myocardial triglyceride content.(18) This may not be surprising,
since high-energy phosphate metabolism was not different between controls and T2DM patients in the first place. In the ZDF rat model, non-thiazolidinedione PPARγ agonist therapy reversed myocardial metabolic derangements, including glucose and fatty acid uptake and triglyceride accumulation.\(^{169}\) The differences between the human and animal study may be due to the difference in severity of the abnormalities, which are much more pronounced in the ZDF rat model as mentioned previously. Another interesting observation in that study,\(^{18}\) was that treatment with the active comparator metformin led to a decrease in cardiac work, paralleled by reduced fatty-acid and glucose metabolism, implying down-regulation of myocardial metabolism in response to less cardiac work. Similarly, cardiac work decreased in parallel to decreased fatty-acid uptake following a 6-week very-low calorie diet in obese otherwise healthy subjects.\(^{170}\) Both the observation of increased fatty-acid metabolism,\(^{25}\) and the ability of a PPARγ agonist to influence myocardial metabolism,\(^{18}\) but also decreased fatty-acid metabolism following a decrease in cardiac work, implicate conservation of metabolic flexibility in human DCM in the non-compromised non-ischemic T2DM heart. Inasmuch as systemic metabolic abnormalities initially lead to myocardial metabolic changes that can be regarded as adaptive as these attempt to overcome the imposed disruption of homeostasis, over time, as diabetes, cardiac and vascular disease progress and interact, these alterations lead to activation of associated pathways that give rise to aberrant biochemical and molecular changes resulting in irreversible damage. Thus, with longer duration of the imposed stress, that was initially compensated for by metabolic adaptive changes, the activated compensatory mechanisms will ultimately become maladaptive, leading to cardiac changes and simultaneous myocardial metabolic inflexibility, which should be regarded as an end-stage fixed condition not amenable for metabolic interventions which is graphically represented in Figure 2.\(^{26}\)

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**Figure 2.** The ‘starling’-curve of myocardial metabolism

Metabolic flexibility is preserved in the early T2DM heart. Initial alterations related to insulin resistant changes in myocardial metabolism \(^1\), only have a minor impact on LV function due to preserved metabolic flexibility \(^2\). However, with disease progression myocardial metabolism and LV function will become increasingly connected as a consequence of reduced cardiac metabolic flexibility. Small changes in metabolic flexibility \(^3\) will then have a disproportionate impact on LV function \(^4\).
Chapter 10

Metabolic interventions in human diabetic cardiomyopathy
Based on the pre-clinical work by Lopaschuk et al. and Unger et al. (94;171) describing the glucolipotoxic phenotype of the T2DM heart, various interventions have been performed addressing the underlying metabolic causes of DCM, (92;169) including intensive glycemic control, lifestyle interventions, consisting of (low-caloric) diets and exercise, PPARy therapy and more recently the novel glucagon-like peptide-1 receptor agonists (GLP-1RA) and metabolic modifiers. Below we describe these interventions with special emphasis on their cardio-metabolic effects.

Intensive glucose control
Several studies have related hyperglycemia to LV diastolic dysfunction. (172;173) Although some studies reported myocardial functional improvements from augmented metabolic control, (174;175) other studies did not show improved function. (172;176) Several large trials on the effect of intensive glucose control (ACCORD trial (n=10251)(177), ADVANCE (n=11140)(178) and VADT (n=1791)(179) trial) did not show a significant reduction in the primary cardiovascular endpoint. In the ACCORD trial overall mortality (↑22%) and CV mortality (↑35%) increased, whereas no significant beneficial effects on overall mortality and CV mortality were seen in de ADVANCE and VADT trial. However, subanalyses of these trials suggested a significant beneficial effect of intensive glycemic control on CVD in participants with shorter diabetes duration, lower HbA1c at inclusion and/or absence of known CVD. (179-181) Although hypoglycaemia and cardiac arrhythmia in these trials have been implicated as causes of increased CV death, an alternative explanation might be that that strict glycemic control led to an ‘unachievable’ metabolic burden on the metabolically compromised heart, which may also explain why intensive glycemic control could be beneficial in the uncompromised heart.

Diet and exercise studies
Several studies have evaluated the effect of dietary interventions on myocardial triglyceride stores. In 14 normoglycemic, non-obese, non-ischemic men, which underwent 31P-MRS, a 3 day very-low calorie diet increased plasma fatty-acids and myocardial triglyceride content, which was correlated with the increase in myocardial triglyceride content, but did not decrease high-energy phosphate metabolism. (182) In a prospective intervention study using 31H-MRS, 10 lean healthy men underwent a three-day partial and complete starvation. (183) Progressive caloric restriction induced dose-dependent changes in myocardial triglyceride content and LV diastolic function. In well-controlled overweight but not obese T2DM patients and without inducible ischemia, a very-low caloric diet increased myocardial triglyceride content and was associated with a decrease in LV diastolic function. (184) When this diet was provided together with the TG-lowering agent acipimox, no changes in myocardial triglyceride content or diastolic function were observed, even though plasma TG decreased. However, a 16-week very-low-calorie diet in obese insulin-treated T2DM patients, resulted in a considerable decrease in body weight associated with a significant improvement of glycemic control. Moreover, myocardial triglyceride content decreased in association with an improvement in diastolic function. (185) Furthermore, in 11 young and lean men performing a 2h cycling protocol, elevations in plasma NEFA, induced by exercise in the fasted state, led to increased myocardial triglyceride content, but did not hamper LV function, although myocardial PCR/ATP decreased. (186) These changes were not observed while subjects ingested glucose during exercise. (186) Also, in 14 middle-aged overweight men, 12 weeks of combined endurance and strength training reduced cardiac triglyceride content, paralleled to a small improvement in EF, without changing body fat and weight. (187) Finally, in 34 obese, otherwise healthy obese subjects
(M/F 10/24), a 6-week very low calorie diet resulted in significant weight loss and decreased PET-measured fatty-acid uptake ([18F-FTHA]), paralleled by reductions in LV mass, cardiac work, resting perfusion and a borderline reduction in myocardial triglyceride content, without alteration in insulin-stimulated myocardial glucose uptake or OF.\(^{170}\) The results and the interpretation thereof in these diet- and exercise studies in healthy subjects are not unequivocal. One may summarize these human studies as follows, 1) myocardial triglyceride content can be manipulated by diet or exercise, occurring as soon as 2h after bicycle exercise, but also after weeks of intervention; 2) The changes in myocardial triglyceride content following non-pharmacological interventions are very modest, at best in the order of an absolute reduction of 1%. The unambiguous finding with regard to LV function and high-energy phosphate metabolism on changes in myocardial triglyceride content from these studies, further spreads doubt about the pathophysiological significance of these marginally altered myocardial triglyceride levels, especially as all these studies were performed in otherwise healthy subjects or in subjects with uncomplicated non-ischemic T2DM patients. Equivalent studies evaluating the effects of long term (chronic) alterations in diet or exercise have so far not been performed. Thus the effects of chronic dietary changes in T2DM remain to be determined.

**Thiazolidinediones**  
After the introduction of the insulin-sensitizing and anti-steatotic PPARy agonists (thiazolidinediones), it was hypothesized that these agents would be the answer to human DCM, as they addressed the presumed key mechanisms underlying this condition.\(^{188}\) Indeed, using troglitazone, cardiac lowering of tri- and diglycerides and ceramide was paralleled by improved contractile function in ZDF rats and decrease of apoptosis.\(^{92}\) Also, treatment of ZDF rats with a non-thiazolidinedione PPARy agonist led to disappearance of myocardial triglycerides increased myocardial glucose uptake, decreased PPARy regulated genes and increased cardiac power.\(^{169}\) After withdrawal from the market of troglitazone due to severe liver toxicity, the 2 novel agents, rosiglitazone and pioglitazone had been introduced successfully as glucose-lowering agents for T2DM, with additional insulin sensitizing, anti-steatotic and anti-inflammatory properties.\(^{188}\) Interestingly, and contrary to their mechanisms of action and the data from animal studies, the use of these agents in humans was associated with increased rates of HF.\(^{189;190}\) Subsequently, it was shown that PPARy activation in the renal tubule lead to fluid retention that could account for the reported increase of clinical CHF.\(^{191}\) Conversely, some short-term small-sized intervention studies in humans indicated that pioglitazone improved LV function in a pre-diabetic hypertensive population. With the publication of the PROACTIVE study,\(^{189}\) showing a reduction in the composite of all-cause mortality, non-fatal myocardial infarction and stroke in T2DM patients with high risk of macrovascular disease, the mechanisms underlying the beneficial effects of pioglitazone still needed further study. The research questions to be asked were whether TZD-related congestive HF was merely due to fluid retention that, when occurring excessively particularly in those with compromised LV, could precipitate a clinical picture of congestive HF and whether TZD's, by manipulating insulin sensitivity and substrate availability, could potentially ameliorate the metabolic conditions for the diabetic heart and hence LV function.\(^{192}\) Several small sized studies were performed and showed improvement,\(^{18;193}\) or no effect on myocardial LV function.\(^{194;195}\) To date, two studies of sufficient size evaluated the effect of the PPARy agonist pioglitazone in relation to its anti-steatotic properties.\(^{195;196}\) In 78 insulin naive T2DM patients without inducible ischemia, randomized to pioglitazone or metformin for 24 weeks, no effect of pioglitazone on myocardial triglyceride content was found, however, myocardial diastolic function and compliance improved.\(^{196}\) The improvement in compliance is a relevant finding, as it suggest that concentric remodeling can be
counteracted by pioglitazone in the uncomplicated, non-ischemic T2DM heart. Indeed, no patient developed pedal edema or HF.(18) In 32 insulin-treated patients with more advanced and poorly controlled T2DM, pioglitazone ad on insulin lowered myocardial triglyceride content, without changing myocardial LV function.(195) Although these studies reveal the potential of pioglitazone to improve LV function or decrease myocardial triglyceride content in humans, they do not directly confirm the animal data suggesting that their anti-steatotic effects are a prerequisite for the improvement of LV function.

**Incretin-based therapies**

Novel agents, based on the incretin glucagon-like-peptide 1 (GLP-1), such as the injectable GLP-1 receptor agonists (GLP-1RA) exenatide and liraglutide and the oral dipeptidyl-peptidase (DPP)-4 inhibitors sitagliptin, vildagliptin and saxagliptin are prescribed to lower blood glucose in T2DM patients.(197) GLP-1 and GLP-1RA therapy results in a sustained glycemic improvement and progressive reduction in bodyweight, which support a shift toward a more favorable cardiovascular risk profile.(197;198) In recent years, cardio-protective properties have been attributed to GLP1-RA from animal models and preliminary patient studies.(199;200) GLP-1RA act through G-protein coupled receptors, which are also present on cardiomyocytes, and raise cyclic AMP.(201) Their effect on LV function and metabolism requires further study; however, infusion of GLP-1 improved cardiac function in animals,(202-204) and patients with CHF.(205-207) Recently, the cardio-protective effects of GLP-1 and its metabolite GLP-1(9-36), which is generated by DPP-4 degradation of GLP-1, were demonstrated in a GLP1-/- mouse model.(208) Thus, the inotropic effects of GLP-1 and its stimulating actions on glucose uptake, ischemic preconditioning and vasodilation were shown to be GLP-1 receptor-mediated, whereas the beneficial effects of GLP-1(9-36) on postischemic recovery of cardiac function are compatible with a GLP-1 receptor-independent action.(208) Liraglutide treatment for 7 days prior to induction of myocardial infarction in C57BL/6 mice reduced cardiac rupture and infarct size and improved cardiac output in parallel with activation of cardioprotective genes, implicating that GLP-1R activation engages prosurvival pathways in mice hearts.(209) Several studies have also reported on the beneficial vascular effects of GLP-1 including amelioration of endothelial dysfunction in T2DM patients with established CAD,(210) a direct vascular action of GLP-1 independently of NO and the endothelium,(211), amelioration of postprandial endothelial dysfunction after a high-fat meal,(212) and controlling post-prandial oxidative stress.(213) Beneficial cardiac effects of DPP-4 inhibitors have also been reported. Sitagliptin reduced infarct size in mice via cAMP-dependent PKA activation.(214) In CAD patients with preserved LV function Sitagliptin improved global and regional LV performance in response to stress and mitigated postischemic stunning in humans with CAD.(215) As GLP-1RA and DPP-4 inhibitors do not cause fluid retention, hypoglycemia or lactic acidosis these drugs may be an interesting option in the treatment of T2DM, especially in vulnerable patients with ischemia or CHF. Large prospective intervention trials in humans applying this novel drug class are eagerly awaited.

**Other metabolic modifiers**

Metabolic modifiers, including perhexiline, trimetazidine, ranozoline and etomoxir decrease myocardial NEFA and increase glucose metabolism by different mechanisms.(216-220) The supposed anti-anginal effect of these agents might be directly due to a rise in myocardial efficiency. Treatment of T2DM patients with CAD with trimetazidine for three-months as compared to placebo improved LV systolic function and functional capacity despite no change in myocardial perfusion.(221) In 19 insulin resistant non-diabetic patients with IDCM, trimetazidine improved EF, paralleled by a
modest decrease in NEFA oxidation, without altering its oxidative rate implying increased oxidation of glucose. (17) In HF patients, three months therapy with etomoxir improved LV function, cardiac output at peak exercise and clinical status. (222) However, there are doubts about the long-lasting safety profile of these metabolic modifiers, which may induce neurotoxicity and/or lipotoxicity (perhexiline) or phospholipodosis (etomoxir).

Bariatric surgery
Bariatric surgery has emerged as an alternative treatment for T2DM patients with a BMI>35 kg/m2 who are difficult to control with lifestyle and pharmacologic therapy. (104) The main mechanisms involved in weight loss and improved blood glucose control with diabetes revolve around increased insulin sensitivity, decreased lipoxicity/inflammation, and changes to gut hormones/incretins. (223-225) Bariatric surgery is associated with a reduced risk of cardiovascular and all-cause mortality, (226) and several studies have shown a beneficial effect of bariatric surgery on LV structure and function and obesity-related co-morbidities. (227-229) Hence, bariatric surgery may be a promising treatment in T2DM patients with DCM, however large scale randomized trials are needed to further explore the long-term effects.

Are metabolic interventions the answer to LV dysfunction in DCM?
As the diabetic epidemic is steadily growing, the search for the ‘ideal’ intervention, possibly a poly-pill, (230) that fulfils all criteria of efficacy and safety and patients acceptance, becomes increasingly relevant. Although exercise and diet, leading to weight loss, are the most efficacious interventions to date and as such generally advocated, it is well know that these lifestyle interventions cannot be sustained by most patients. TZD treatment may be beneficial in the uncomplicated, non-ischemic T2DM heart. However, due to the increased incidence of TZD related HF, TZDs should not be used in the compromised heart. Currently, convincing data on the cardiovascular efficacy and safety of the incretin-based therapies and metabolic modifiers in DCM are lacking but large outcome trials are underway (clinicaltrials.gov), among others sitagliptin (TECOS), lixisludide (LEADER), exenatide (EXCEL), alogliptin (EXAMIN) and linagliptin (CAROLINA). Although promising with respect to their mechanism of action, with the disconcerting state of affairs related to rosiglitazone, (231) large dedicated randomized controlled trials are needed before any of these treatments should be initiated as regular therapy in DCM.

Summary and conclusions
The definition of DCM as a distinct disease entity by Rubler et al, (27) has generated abundant research in the field over the past three decades. In spite of these efforts, to date, many questions remain unanswered concerning the etiology of human DCM, since the mechanisms that emerged from animal studies only partly seem to apply in humans. Based on the present insights, DCM should be viewed not as a single condition but rather as part of a complex heterogeneous cardiometabolic syndrome that develops and evolves over time in interaction with concomitant complications and co-morbidities. In the present review we have substantiated the need for dedicated research in human DCM to address the mechanisms obtained from rodent models of DCM. Over the last two decades non-invasive functional assessments with echocardiography and MRI have been introduced, simultaneously with the development of dedicated PET and MR spectroscopy techniques which has enabled phenotyping cardiac function and metabolism in human DCM. In the present review we confirm that both functional and metabolic changes are part of human DCM, however, these are not intimately connected in all stages of DCM, particularly as in the early
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Phase of DCM the heart possesses adequate metabolic flexibility to counteract the imposed metabolic stress. We, however, reason that with longer duration of the imposed stress, the activated compensatory mechanisms will ultimately become maladaptive, which leads to cardiac changes and simultaneous myocardial metabolic inflexibility, which should be regarded as an end-stage fixed condition not amendable for metabolic interventions. Metabolic interventions to treat DCM may therefore be beneficial in early DCM, but detrimental in late DCM. Initiation of metabolic interventions to delay and ultimately prevent cardiac functional deterioration should be directed at strategies taking into account the changing DCM phenotype over time, which may develop differently between gender and race, and the occurrence of additional stressors such as hypertension and microvascular disease.
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