FINAL PROGRESS REPORT
Diabetes Complications Consortium Grant: **"A Murine Model of Diet-Induced Insulin Resistance and Cardiomyopathy"**
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This serves as a final progress report for our Diabetes Complications Consortium (DCC) grant, entitled, "A Murine Model of Diet-Induced Insulin Resistance and Cardiomyopathy." We proposed determining whether either of two diets, a "diabetogenic diet" (DD) or a diabetogenic diet with added cholesterol (DDC) could induce cardiac hypertrophy and fibrosis in wild-type, black and tan brachyuric (BTBR) mice. We successfully completed Aims 1 and 3, and augmented Aim 1 by adding two intervention arms, designed to determine whether either adding metformin to the diabetogenic diet (DD) or changing to Chow diet could reverse DD-induced cardiomyopathy. To date, these studies have resulted in **SIX** abstracts that have been presented at regional meetings (N=3) and at national meetings (N=3) [1 at the American Heart Association, published in ***Circulation***; 3 at the Western Society for Clinical Investigation, published in the ***Journal of Investigative Medicine***; and 2 at the Heart Failure Society of America (HFSA), published in the ***Journal of Cardiac Failure***]. One of the abstracts accepted by HFSA was among only six selected for presentation in the HFSA **Featured Oral Abstract Session**. *We sincerely thank the DCC for funding these studies*.

Results are presented for each Hypothesis of the original application.

Aim 1. Validate the AMDCC criteria of cardiac hypertrophy, increased fibrosis and myocyte size with DD and DDC diets in the BTBR mouse model.

1. Confirm that DD and/or DDC induce cardiomyopathy, and determine the timecourse of cardiomyopathy development. At 4 weeks of age, female BTBR mice were placed on either standard rodent chow ("Chow") or a "diabetogenic" diet with 59% of calories from fat and 26% of calories from carbohydrates ("DD"). Oral glucose tolerance tests were performed at 8 and 16 weeks on diets. Mice were sacrificed after 16 and 30 weeks on diets for determination of heart weights and fibrosis. Final group sizes were 7-8 mice/diet group for each timepoint.

We found that, by 8 weeks on DD, female BTBR mice had normal fasting glucose, but glucose tolerance (as judged by area under the glucose excursion curve) was significantly impaired vs. Chow-fed controls. At 16 weeks on diets, blood pressures did not differ significantly, but fasting glucose was increased by 22% (P=0.0125), AUC by 23% (P=0.003), heart weights 18% (P<0.001), and LV cross-sectional area by 13% (P=0.020) in DD as compared to Chow mice. At 30 weeks on diets, mean fasting glucose was increased by 21% (P=0.0034), AUC was increased by 27% (P=0.023), heart weights increased by 20% (P<0.001), and LV cross-sectional ared increased by 18% (P=0.0049) in DD as compared to Chow mice. Though fibrosis, as measured by picrosirius stain, was increased by 26% at 16 weeks and by 23% at 30 weeks in DD vs. Chow, these differences did not reach statistical significance. The major findings of these studies are summarized in **Table 1**.

Table 1. Summary of Time Course of DD-induced Cardiomyopathy in Wild-type BTBR mice. Shown are relative changes with DD vs. Chow in body weight, dysglycemia measures [glucose and area under the oral glucose tolerance curve (AUC)], cardiac hypertrophy (heart weight and LV cross-sectional area), fibrosis (by Masson's trichrome stain) and inflammation (quantitative immunohistochemistry for macrophages) at 8, 16 and 30 weeks on diets. ***P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.**

	% Δ, DD vs. Chow		
Diet Duration (Wks)	8	16	30
Body Weight	**14%****	**24%*****	**64%******
Glucose	3%	**22%***	**21%***
AUC	**23%****	**23%****	**24%***
Heart Weight	--	**18%******	**20%******
LV Cross-sectional Area	--	**13%***	**18%****
Fibrosis	--	**197%*****	NS
Inflammation	--	NS	NS

Therefore, we concluded that:

- 16 weeks feeding of a "diabetogenic" diet to wild-type, female BTBR mice results in significant dysglycemia and myocardial hypertrophy.
- Increasing the length of diet treatment to 30 weeks does not further worsen dysglycemia or the cardiomyopathy phenotype.

- These findings suggest that feeding a diabetogenic diet to wild-type BTBR mice for 16 weeks is sufficient for studying the pathogenic mechanisms underlying cardiomyopathy in insulin resistance and diabetes.
- In addition, we found that addition of 0.15% cholesterol to DD (a "DDC" diet), induced cardiomyopathy that was similar to that induced by DD (i.e., similar increases in cardiac hypertrophy and fibrosis). Therefore, we concluded that it was not necessary to further test DDC.

2. Determine whether addition of Metformin or switch to Chow diet for can induce regression of established cardiomyopathy. Because (DD) to wild-type BTBR mice for 16 weeks induces significant dysglycemia and cardiac hypertrophy, we hypothesized that either treatment with metformin or switching to a Chow diet would favorably affect body weight, dysglycemia and cardiomyopathy in this model. At 4 weeks of age, female BTBR mice were placed on a diabetogenic diet, with 59% of calories from fat and 26% of calories from carbohydrates (DD). At 20 weeks on DD, mice were placed into one of three groups (N=7-8/group) for 10 additional weeks: 1) continued DD (DD group), 2) continued DD plus Metformin treatment (100 mg/kg/day) (DD+Met group), and 3) switched to Chow diet (Regression group). Dysglycemia was assessed by fasting glucose levels and oral glucose tolerance tests (expressed as area under the glucose excursion curve, or AUC) obtained prior to sacrifice. Cardiac hypertrophy was assessed by measurement of wet heart weights, and fibrosis using trichrome stain.

While blood pressures were similar across all groups, both interventions resulted in significant decreases body weight vs. DD, with decreases of 16% (P=0.024) with DD+Met and of 31% (P=0.003) with Regression diet. Fasting glucose was decreased significantly in the DD+Met group (-22%; P=0.034) but not in the Regression group (-6%, P=0.24) vs. the DD group. Cardiac hypertrophy was not significantly different in either DD+Met (-6%, P=0.08) or Regression (-2%, P=0.69) groups as compared to the DD group. However, fibrosis was decreased significantly in the DD+Met group (-29%, P=0.030), but not in the Regression group (-4%, P=0.79).

Table 2. Summary of Intervention Studies. Shown are the effects of 10 weeks of Metformin added to DD or of change from DD to Chow diet after 20 weeks of DD in body weight, dysglycemia measures [glucose and area under the oral glucose tolerance curve (AUC)], cardiac hypertrophy (heart weight and LV cross-sectional area), fibrosis (by Masson's trichrome stain) and inflammation (quantitative immunohistochemistry for macrophages). ***P<0.05, **P<0.01.**

	%Δ, DD+Met vs. DD	% Δ, DD→Chow vs. DD
Body Weight	NS	**-31%****
Glucose	**-22%***	NS
AUC	NS	NS
Heart Weight	NS	NS
LV Cross-sectional Area	NS	NS
Fibrosis	**-35%***	NS
Inflammation	NS	NS

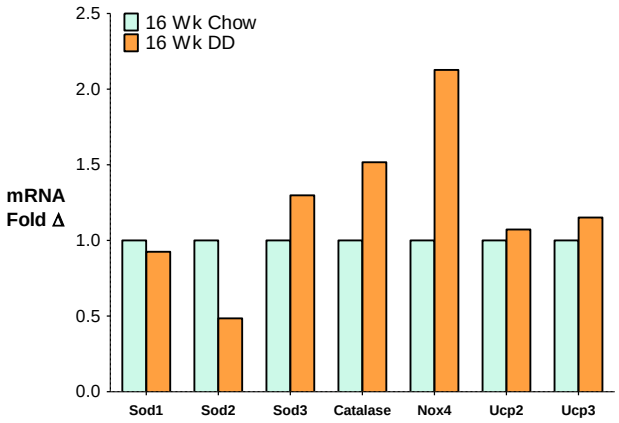
Therefore, we concluded that:

- Despite significant improvements in body weight, neither addition of metformin to a diabetogenic diet nor switching to chow diet affected cardiac hypertrophy.
- However, both fasting glucose and cardiac fibrosis decreased significantly with metformin
- Surprisingly, aggressive dietary intervention with the regression diet had no significant effect on either endpoint.
- Whether earlier intervention with metformin and/or dietary change might also blunt the development of cardiac hypertrophy in this model warrants further study.

Aim 2. Validate the additional AMDCC criterion of cardiac dysfunction. We were not able to perform these studies, as our co-investigator had lost the underlying funding to support the core laboratory that we planned to use for these measurements. Instead, we added the dietary and metformin intervention studies described under Aim 1.

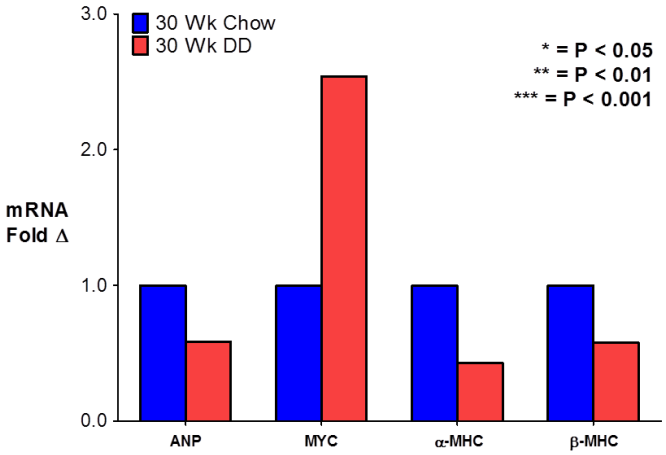
Aim 3. Perform preliminary studies of the roles of specific molecular pathways in disease development. We were able to document, by quantitative reverse transcription/polymerase chain reaction (qPCR), interesting changes in several oxidant stress genes after 16 weeks on DD vs Chow diets (Fig. 1). The increase in *Nox4* expression

Figure 1. Comparison of <u>oxidant stress</u> gene expression levels by qPCR at 16 weeks on Chow vs. DD diets in wild-type BTBR mice. qPCR was performed for superoxide dismutases (Sods) 1-3, catalase, NAPDH oxidase (Nox4) and uncoupling proteins (Ucps) 2 and 3. <u>Nox4</u> expression was increased >2-fold in DD-fed mice (**P<0.05**). The approximately 50% decrease in Sod2 expression with DD did not reach statistical significance.



Perhaps more interesting, we also performed qPCR analyses comparing expression of several "hypertrophy" gene markers after 30 weeks on Chow vs. DD diets, finding that Myc was significantly elevated (Fig. 2). Myc recently has been implicated in transcriptional regulation of cardiac metabolism and mitochondrial biogenesis in response to pathological stress in mice (Ahuja P, *J Clin Invest* 120(5): 1494-1505, 2010).

Figure 2. Comparison of <u>hypertrophy</u> gene expression levels by qPCR at 30 weeks on Chow vs. DD diets in wild-type BTBR mice. qPCR was performed for atrial natriuretic peptide (ANP), Myc, α- and β- myosin heavy chains (MHCs). Myc expression was increased >2.5-fold (P<0.05), while none of the other changes in gene expression reached statistical signficance



The following **Abstracts** were published as a result of funding from this DDC grant:

1. Sta Teresa A, Kim J, Wietecha T, Hudkins-Loya K, Alpers CE, **O'Brien KD**. A murine model of diet-induced insulin resistance and cardiomyopathy. ***Circulation*** 124:A18296, 2011.
2. Sta Teresa A, Kim J, Wietecha T, Hudkins K, Alpers C, **O'Brien KD**. Time course of development of a murine model of diet-induced insulin resistance and cardiomyopathy. ***J Investig Med*** 60(1):133, 2012.
3. Kim J, Wietecha T, Sta Teresa A, Hudkins K, Alpers C, **O'Brien KD**. Metformin improves obesity and fasting glucose, but not established cardiac hypertrophy, in a murine model of diet-induced insulin resistance and cardiomyopathy. ***J Investig Med*** 60(1):133-134, 2012.
4. Wietecha T, Kim J, Sta Teresa A, Hudkins K, Alpers C, **O'Brien KD**. Dietary regression reduces body weight but not dysglycemia or established cardiac hypertrophy and fibrosis in a murine model of diet-induced insulin resistance and cardiomyopathy. ***J Investig Med*** 60(1):134, 2012.
5. Wietecha T, Kim J, Sta Teresa A, Hudkins K, Alpers CE, **O'Brien KD**. Metformin, but not diet-induced weight loss, decreases myocardial fibrosis in a murine model of diet-induced insulin resistance and cardiomyopathy. ***J Card Fail*** 18(8):S7, 2012. (**<u>Featured Oral Abstract Presentation</u>**).
6. Sta Teresa A, Kim J, Wietecha T, Hudkins K, Alpers CE, **O'Brien KD**. 16 weeks of diabetogenic diet are sufficient to induce cardiac hypertrophy and fibrosis in a murine model of diet-induced insulin resistance and cardiomyopathy. ***J Card Fail*** 18(8):S22, 2012.