Bromocriptine-QR Ameliorates a Pro-oxidative stress / Pro-inflammatory Monocyte Phenotype in Poorly Controlled T2DM Subjects

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Disclosure

Anthony H. Cincotta is the President and Chief Science Officer and share holder of VeroScience

Michael Ezrokhi and Nicholas Cominos are employees of VeroScience

VeroScience developed Bromocriptine-QR (Cycloset),
a therapy approved by FDA to treat Type 2 Diabetes

Eugenio Cersosimo, John Adams, Mariam Alatrach, Christina Agyin,
Curtis Triplitt, and Ralph A. DeFronzo have no conflict of interest
related to this study

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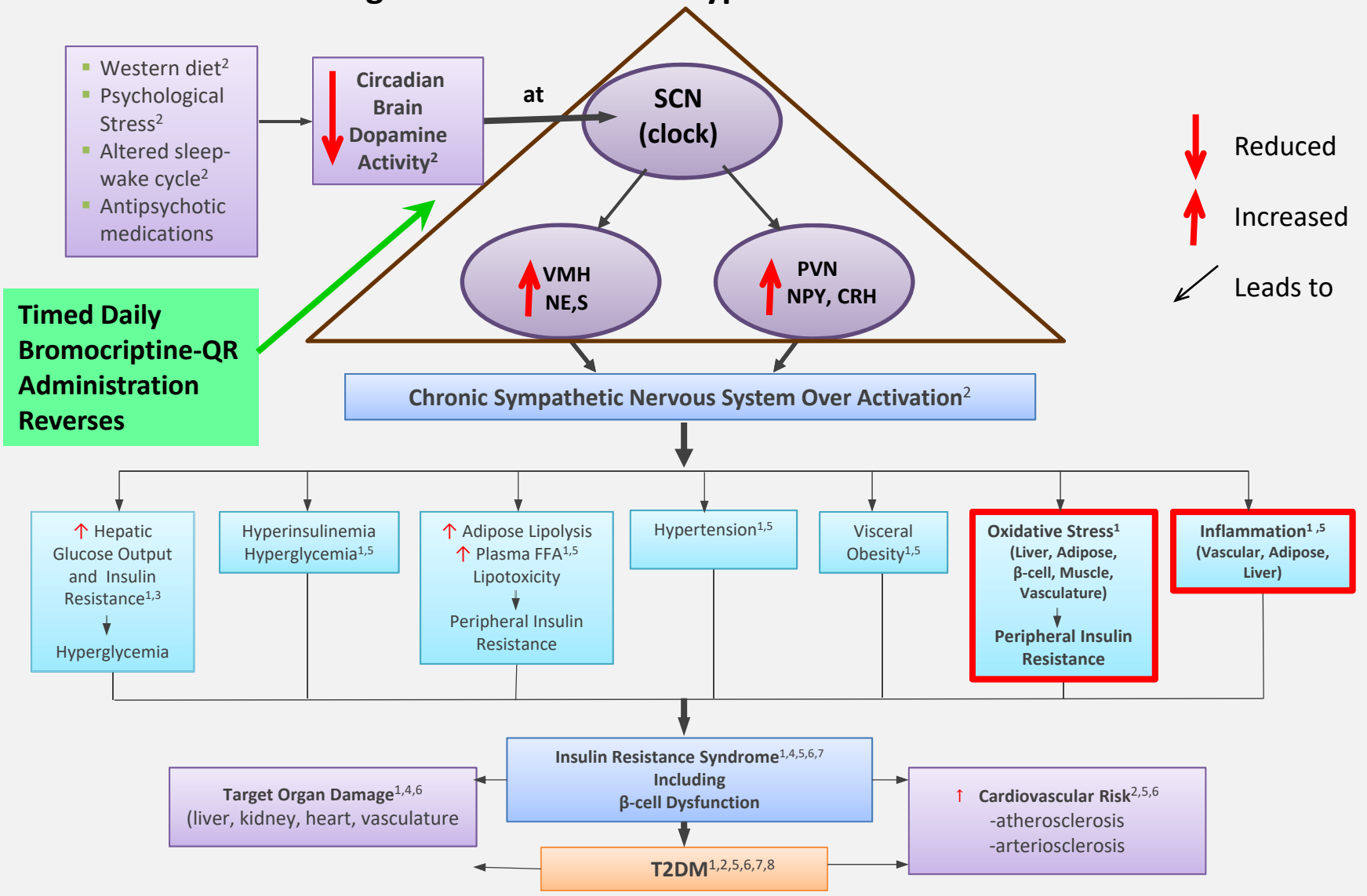
Reduction of Normal Daily Peak in Hypothalamic Dopamine Activity Potentiates the Progression of CVD and Type 2 Diabetes

Introduction



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Design

Background

- Elevated sympathetic tone stimulates a pro-oxidative stress / pro-inflammatory state systemically and in immunocytes that induces and potentiates insulin resistance, dysglycemia, and cardiovascular disease
- Bromocriptine-QR is a sympatholytic dopamine agonist FDA-approved for treatment of T2DM that improves insulin resistance and postprandial dysglycemia and reduces cardiovascular events.
- Timed daily Bromocriptine-QR administration has been demonstrated to reduce several metabolic parameters of the insulin resistance syndrome in various animal models and in humans.

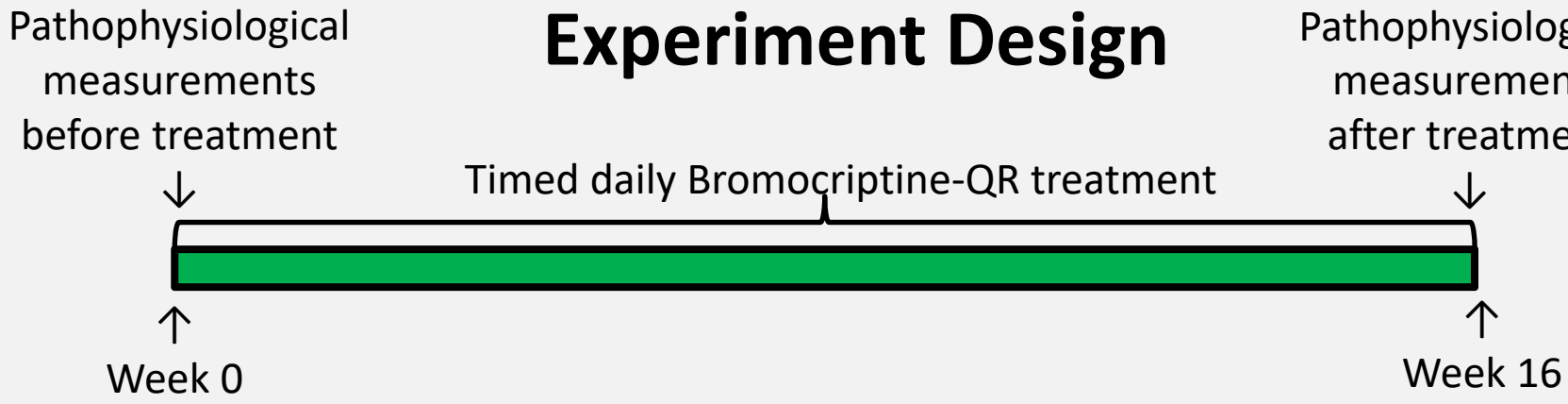
Study Objective

To examine the effect of timed daily Bromocriptine-QR on the pro-oxidative stress / pro-inflammatory phenotype of blood monocytes in T2DM subjects

Model System

Primary blood mononuclear cells are easily accessible in a clinical trial setting and in T2DM are known to exhibit a pro-oxidative stress / pro-inflammatory phenotype that contributes to insulin resistance and CVD. Peripheral blood transcriptome dynamically reflects system wide biology and has been shown to reflect effects of diet as well as changes in response to pharmaceutical intervention respecting inflammatory state [Boss A et al. Int J Mol Sci. 2016;17(12):2019; Liew C et al. J. Lab. Clin. Med. 2006, 147, 126–132; Olsen K et al. Curr. Nutr. Rep. 2015, 4, 377–386].

Experiment Design



Study Design

- Fifteen Type 2 diabetic patients whose hyperglycemia was poorly controlled with a stable dose of metformin plus GLP-1 receptor agonist received timed daily Bromocriptine-QR (3.2 mg/day) for 16 weeks.
- Pathophysiological metabolic parameters (meal intolerance, dysglycemia, endothelial dysfunction, hypertension), were measured and blood samples were collected at baseline and after 16 weeks of treatment.
- Plasma samples were tested for oxidative stress markers, pro-inflammatory cytokines, and sympathetic tone markers.
- Blood monocytes were isolated and gene expression was quantified by RT-qPCR for the following genes: (i) master antioxidant gene regulators that increase in response to systemic oxidative stress (OS) (oxidation resistance gene 1 [OXR1] and nuclear factor erythroid 2 like 2 [NRF2]); (ii) genes known to induce a proinflammatory profile in T2DM (toll like receptor 2 [TLR2], nuclear factor kappa B p65 [NFkBp65], and L-selectin, a surface adhesion protein that stimulates monocyte trans-endothelial migration and proinflammatory biochemistry); (iii) glucocorticoid receptor [GCR] that is elevated in response to a proinflammatory environment.

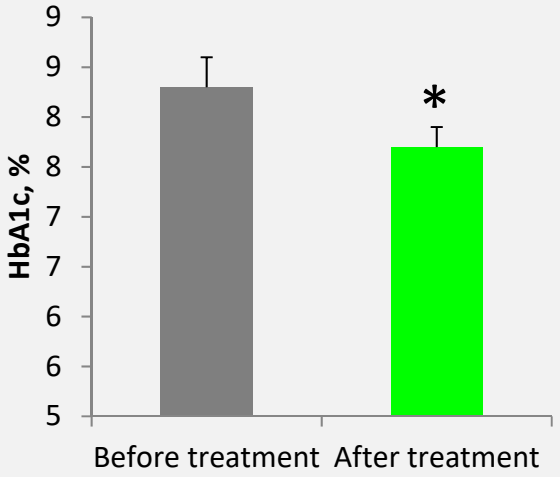
Materials and Methods

- Endothelial function was measured by post-occlusion hyperemia.
- Gene expression was measured by qPCR in the mononuclear cell samples collected before and after Bromocriptine-QR treatment, before mixed meal test
- 15 subjects participated in the study, 14 subjects had the samples collected
- Total RNA was isolated with Trizol reagent; total RNA concentration in all samples was determined by UV spectrophotometry, and adjusted prior to DNAse / reverse transcription steps. All qPCR was performed with TaqMan probes method, using ThermoFisher primer/probe kits with the exon-spanning probes. All qPCR values were normalized to RPS17 (Ribosomal Protein S17)
- Plasma oxidative stress markers, pro-inflammatory cytokines, and sympathetic tone markers were determined by ELISA assays using Cayman, Legend BioScience, and Alpco kits.

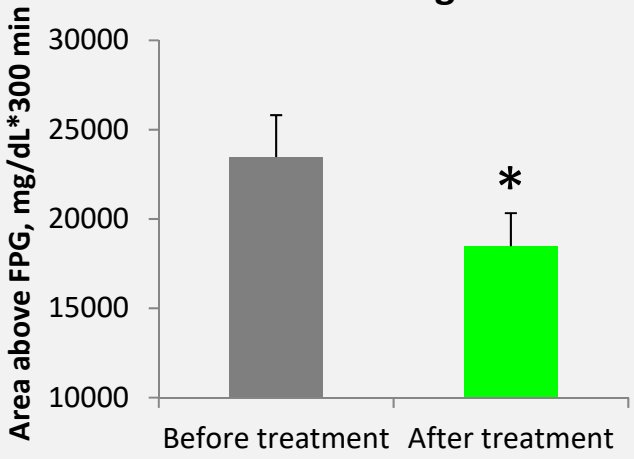
Timed daily Bromocriptine-QR treatment significantly improved HbA1c, postprandial hyperglycemia, blood pressure, and endothelial dysfunction

Results

HbA1c

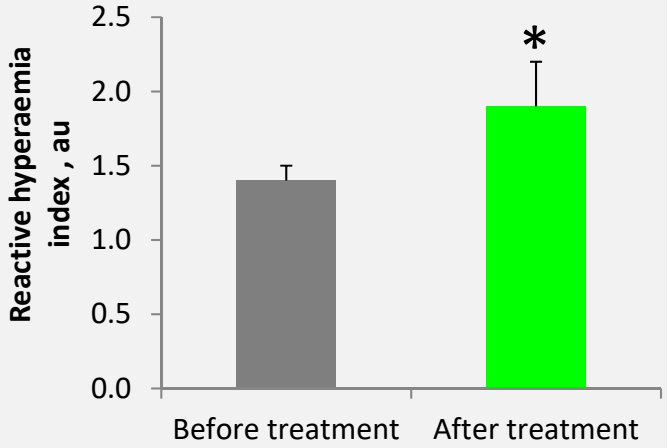


Incremental Area Above the Fasting Plasma Glucose Concentration During MMT

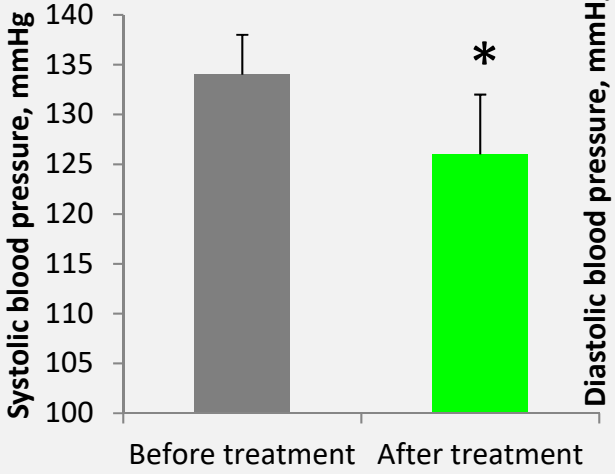


Reactive Hyperemia Index

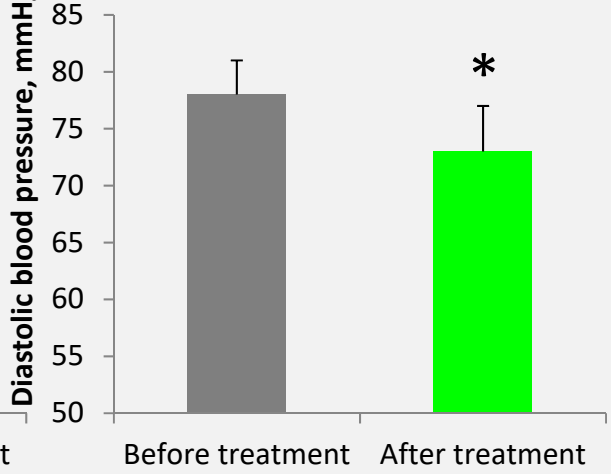
(endothelial function assessment)



Systolic Blood Pressure

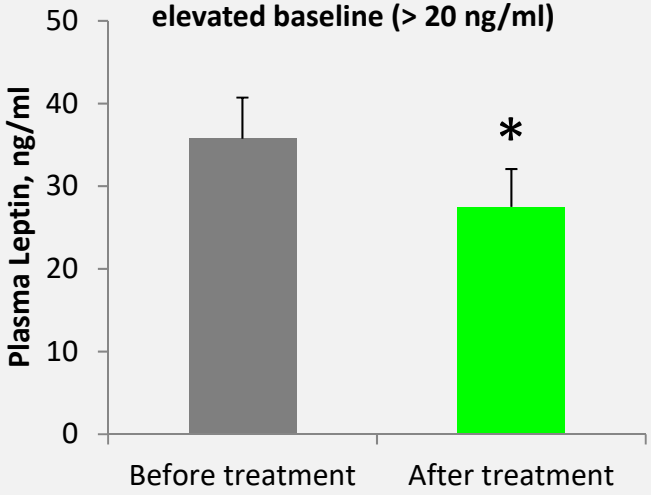


Diastolic Blood Pressure



Plasma Leptin

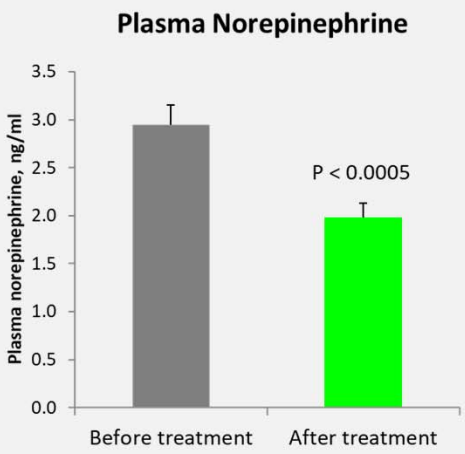
elevated baseline (> 20 ng/ml)

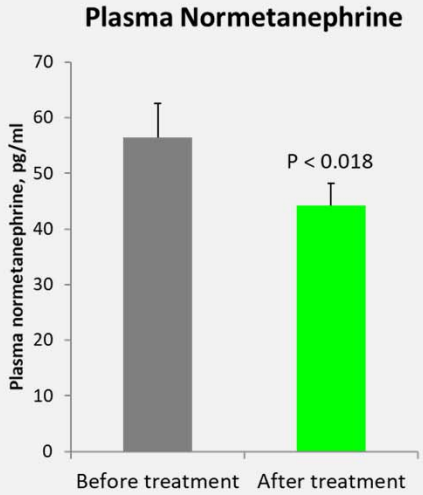


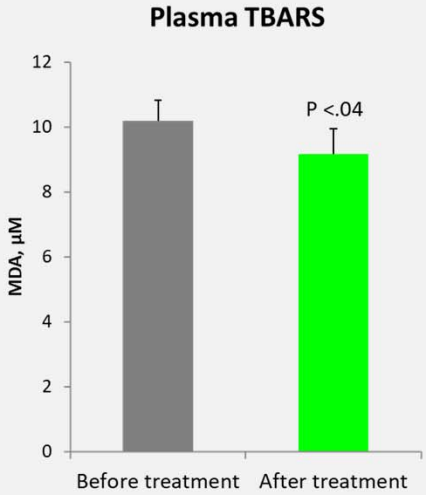
MMT: mixed meal tolerance test * Difference in the measured parameter before and after Bromocriptine-QR treatment was significant (P<0.05)

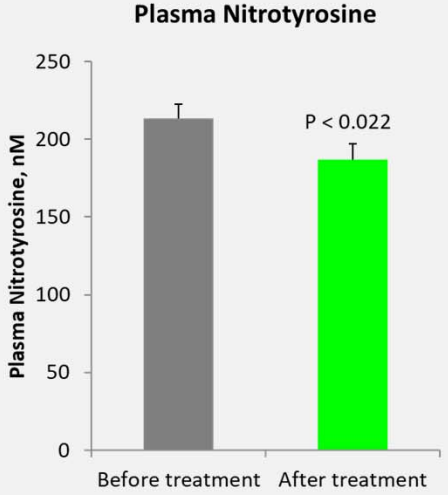
Results

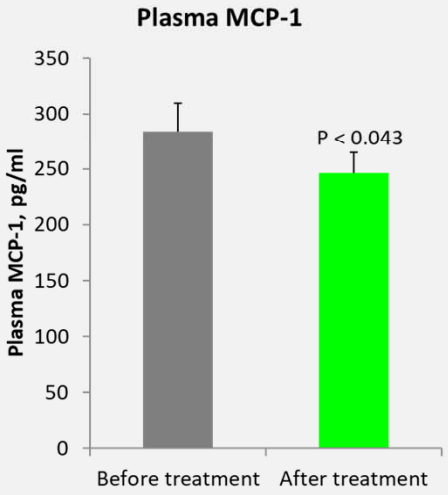
Plasma levels of oxidative stress markers (nitrotyrosine and TBARS), pro-inflammatory cytokines (MCP1, IL-6 and IL-18) and sympathetic activity markers (norepinephrine and normetanephrine) were significantly reduced following 16 weeks of timed daily Bromocriptine-QR treatment

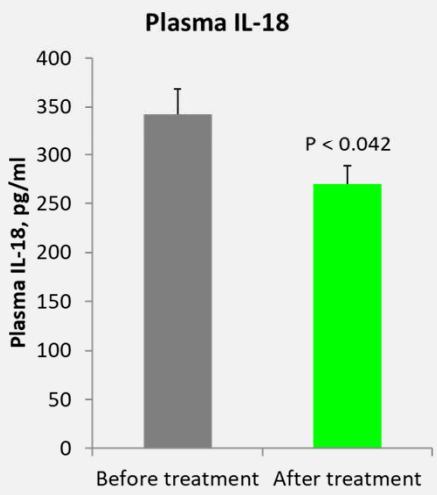


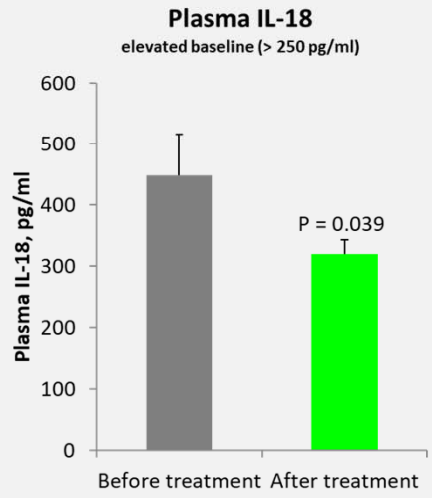


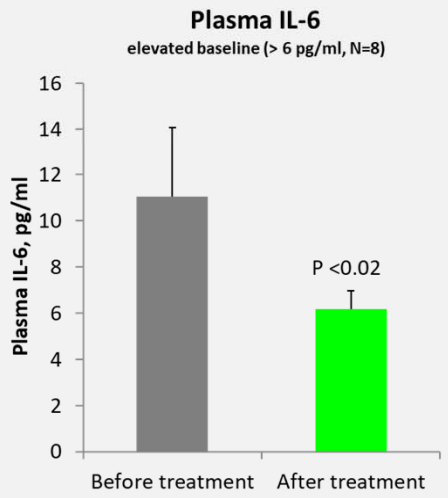






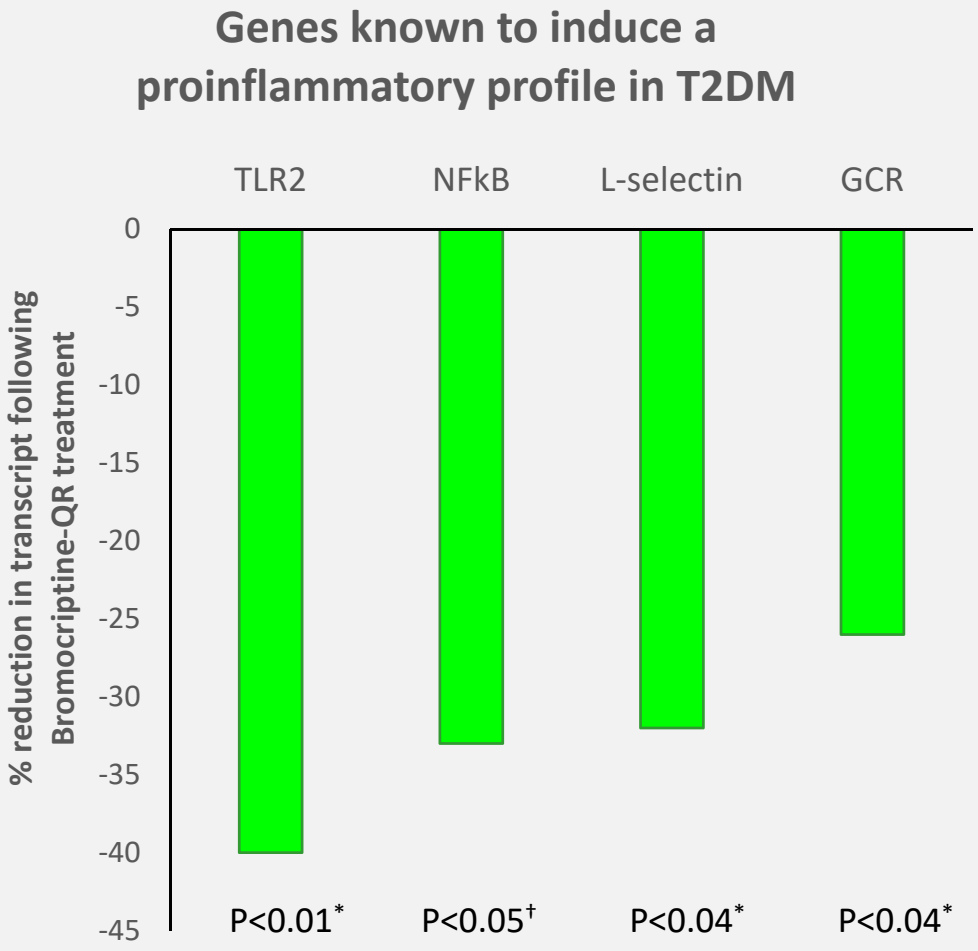






Genes known to induce or respond to a pro-inflammatory profile in type 2 diabetes mellitus were significantly reduced following 16 weeks of timed daily Bromocriptine-QR treatment

Results



TLR2: Toll Like Receptor 2, known to activate IκB kinase that leads to activation of the NFkB pro-inflammatory pathway

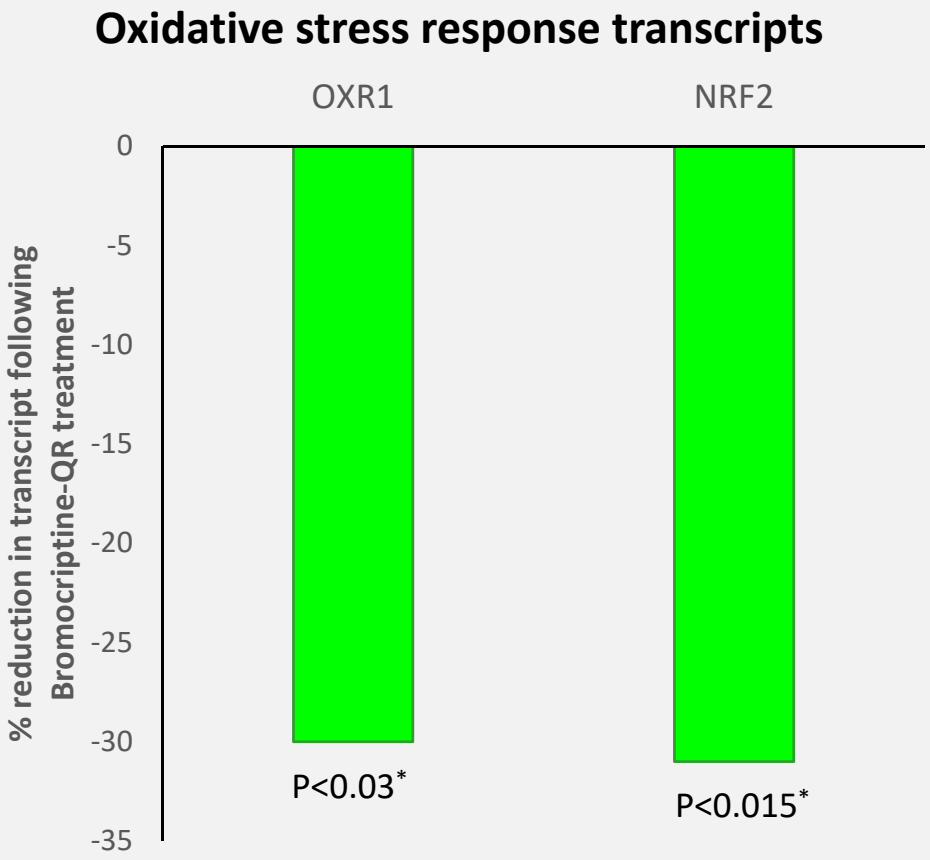
NFkB: Transcription factor p65 also known as nuclear factor NF-kappa-B p65 subunit, induces expression of proinflammatory genes including cytokines, chemokines, and adhesion molecules

L-selectin: L-selectin, also known as CD62L, is a surface adhesion protein that stimulates monocyte trans-endothelial migration and proinflammatory biochemistry

GCR: Glucocorticoid receptor (GR, or GCR) also known as NR3C1, that is elevated in response to a proinflammatory environment

* Difference in mRNA level in mononuclear cells before and after Bromocriptine-QR treatment was significant (2-tailed paired t-test)
† Difference in mRNA level in mononuclear cells before and after Bromocriptine-QR treatment was significant (1-tailed paired t-test)

Oxidative stress response gene transcript levels of OXR1 and NRF2 in mononuclear cells were significantly reduced following timed daily Bromocriptine-QR treatment



OXR1 and NRF2 are master antioxidant gene regulators that increase in response to systemic oxidative stress. OXR1 and NRF2 are elevated in oxidative stress conditions

Reduction in OXR1 and NRF2 expression is consistent with oxidative stress reduction following timed daily Bromocriptine-QR treatment

OXR1: Oxidation resistance gene 1. Human OXR1 genes are induced in response to oxidative stress. OXR1 gene was reported to regulate the expression of ROS detoxification enzymes. OXR1 is thought to play a role in antioxidant defense regulation.

NRF2: Nuclear factor erythroid 2-related factor 2 (NRF2), also known as nuclear factor erythroid-derived 2-like 2. Nrf2 is a transcription factor that plays an important role in cellular defense against oxidative stress by upregulating the expression of antioxidative enzymes.

* Difference in mRNA level in mononuclear cells before and after Bromocriptine-QR treatment was significant (2-tailed paired t-test)

Conclusion

Timed daily administration of Bromocriptine-QR in T2DM patients whose hyperglycemia was poorly controlled with metformin plus GLP-1 receptor agonist

- Reduces postprandial dysglycemia
- Improves endothelial function
- Reduces blood pressure
- Reduces levels of vascular oxidative stress markers
- Reduces circulating monocyte pro-oxidative/pro-inflammatory state

These improvements in T2DM pathophysiology provide a potential mechanism for timed daily Bromocriptine-QR's cardiovascular protective effect

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A large randomized cardiovascular outcomes trial demonstrated that Bromocriptine-QR significantly reduced a prespecified composite cardiovascular endpoint in T2DM subjects. 75% of the study subjects did not have preexisting cardiovascular disease at baseline.

(Diabetes Care 2010, 33(7) 1503-08; J Am Heart Assoc. 2012 Oct;1(5):e002279; J Diabetes Res. 2015;2015:157698; Postgrad Med. 2016 Nov;128(8):761-769; Endocrinol Diabetes Metab. 2019 Nov 13;3(1):e00101)