ENHANCEMENT OF BROWN ADIPOSE TISSUE DEVELOPMENT *IN VIVO* BY A NOVEL INSULIN SENSITIZER

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Presenter Disclosure

Jerry R. Colca, PhD

Board Member/Cofounder: **Metabolic Solutions Development Co., LLC**

Employee: **Metabolic Solutions Development Co., LLC**

Stock/Shareholder: **Metabolic Solutions Development Co., LLC**

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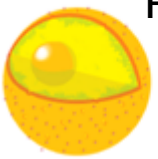
Mechanism of Action for Insulin Sensitizers

Old

Troglitazone; Rosiglitazone; Pioglitazone

Original TZDs



PPAR-Driven Gene Changes

Fat sequestered
Increased Insulin Action
Fluid Retention
Weight Gain

New

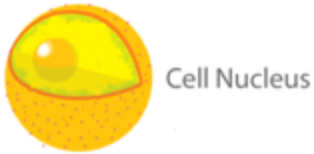
MSDC

Mito Target of TZDs (mTOT)

MSDC-0160
MSDC-0602

(Phase 2 clinical trials)

Metabolic signals



Nuclear Regulatory Factors

Improved Insulin Action
Improved Lipid Profiles

Increased Brown Fat
Preservation of β-cells

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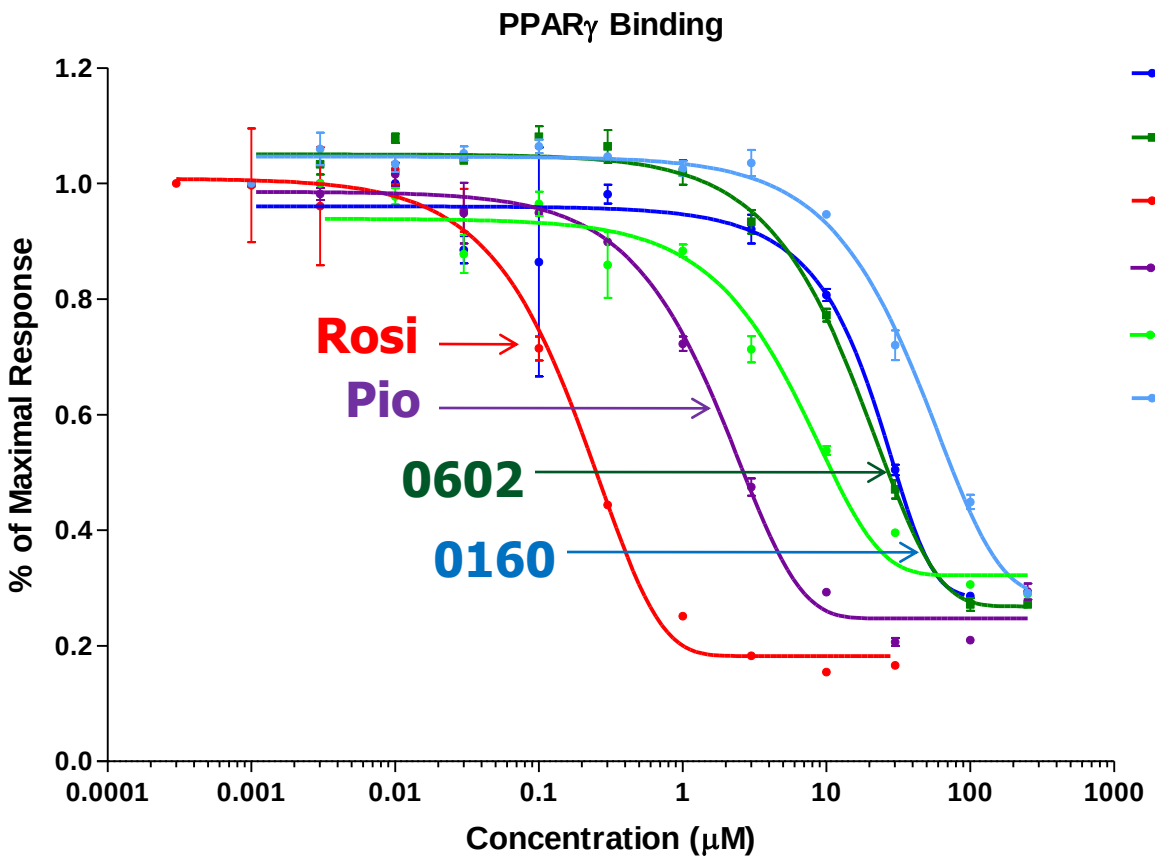
PPARγ Sparing Clinical Candidates



		Binding	Activation
	EC_{50}(μM)	Fold pio[1]	Fold pio[2]
Pio	1.623	1.00	1.00
602	15.54	**9.57**	**12.96**
160	23.73	**14.62**	**9.58**
53	45.22	27.86	26.58
597	6.15	3.79	3.50

[1] Lantha screen –binding
[2] Gene blazer- cell activation

- Pioglitazone is less PPARγ activating than rosiglitazone
- Compounds can be identified that are significantly less PPAR activating

- **PPAR-sparing compounds are able to increase brown adipose tissue in a PPAR-independent manner.**

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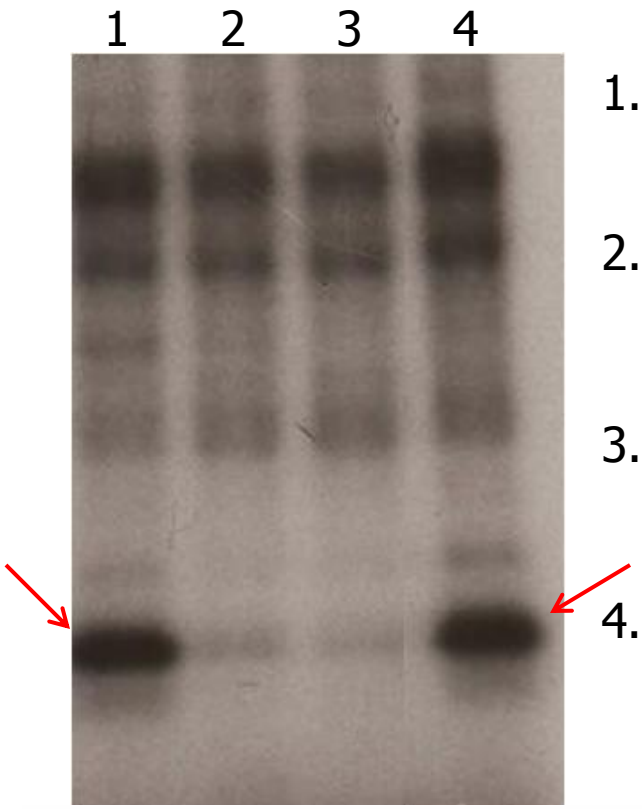
mTOT Mitochondrial Target of TZDS

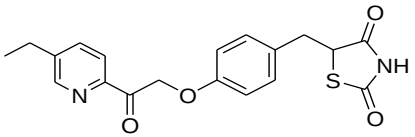
Compounds that compete increase UCP1 expression

Photoaffinity crosslinking



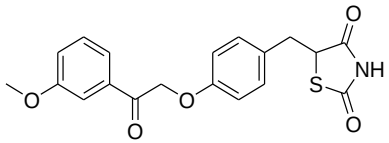
1. DMSO

2.



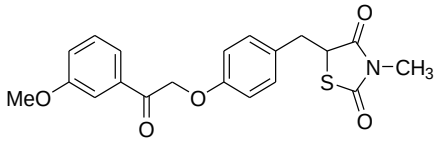
MSDC-0160

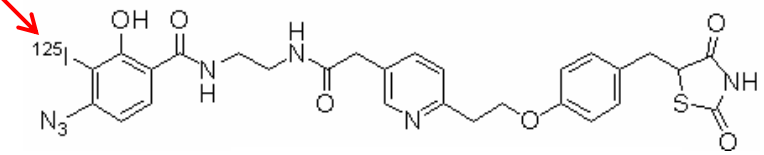
3.



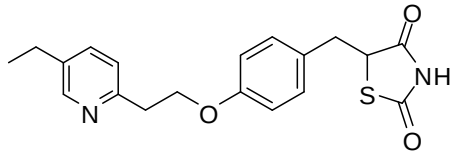
MSDC-0602

4.

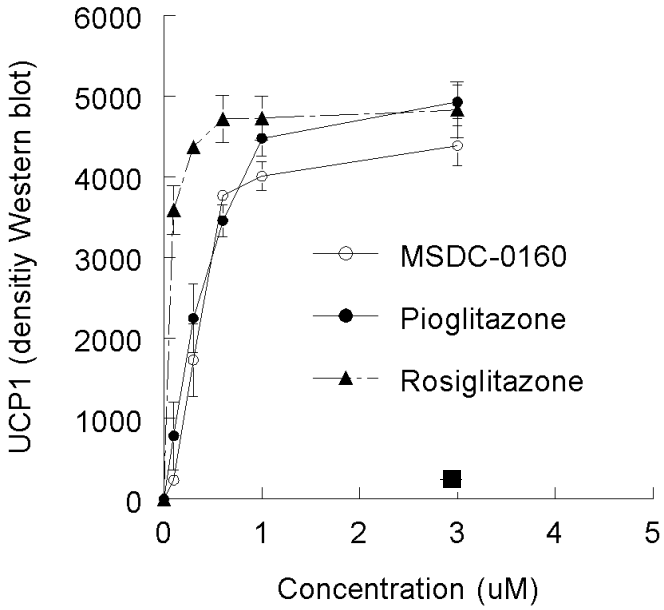


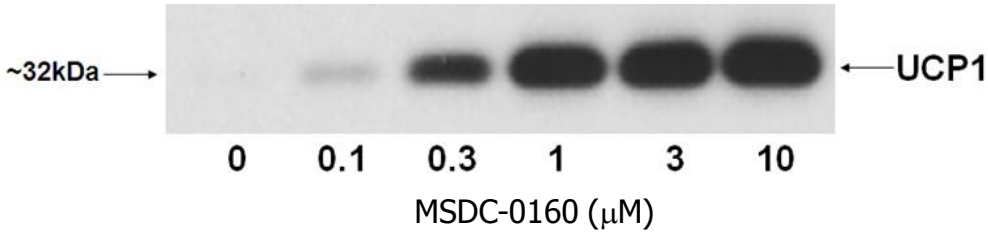


Pioglitazone



UCP1 Western Blot





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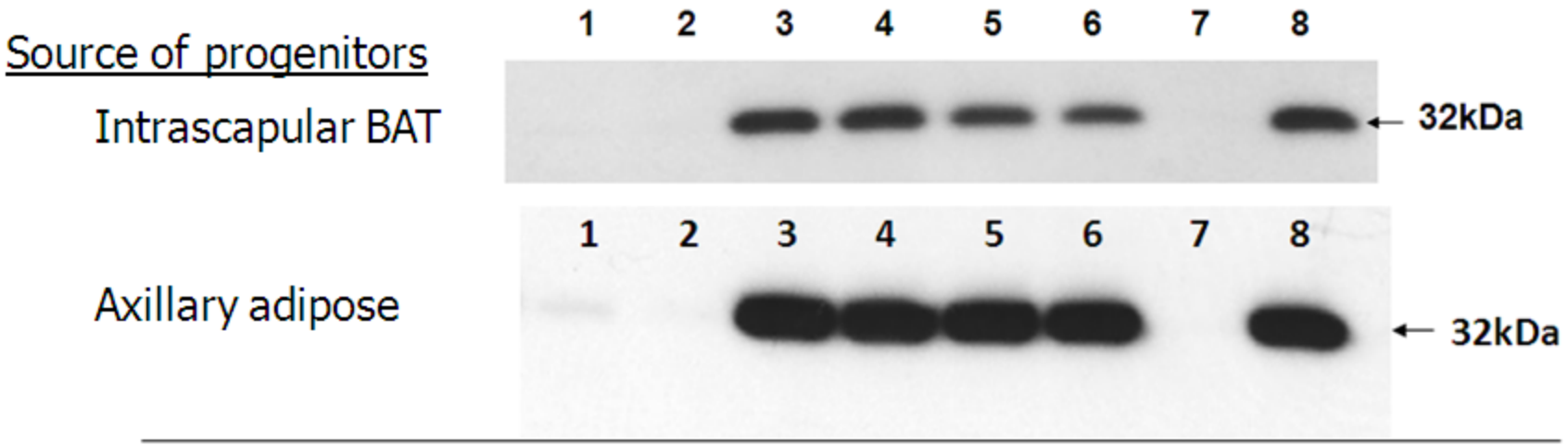
Increase in UCP1 Protein is Not Blocked By PPARγ Antagonists – BAT and Axillary Progenitors

Pre-treatment with or without compounds and antagonist for 6 days



T0070907 (1 μM)	-	+	-	-	+	+	-	-
Rosi (0.3 μM)	-	-	+	-	+	-	-	+
MSDC-0160 (1 μM)	-	-	-	+	-	+	-	-
GW9662 (1 μM)	-	-	-	-	-	-	+	+

See also poster **1603P** Bill McDonald, *et al .* Novel Insulin Sensitizers Enhance Brown Adipose Cell Differentiation by Modulation of the Wnt Signaling Pathway

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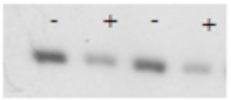
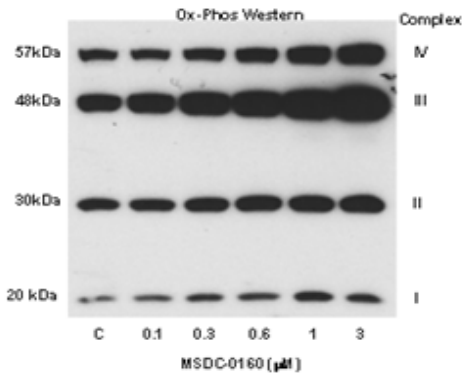
Time Course of Effects *In Vitro*

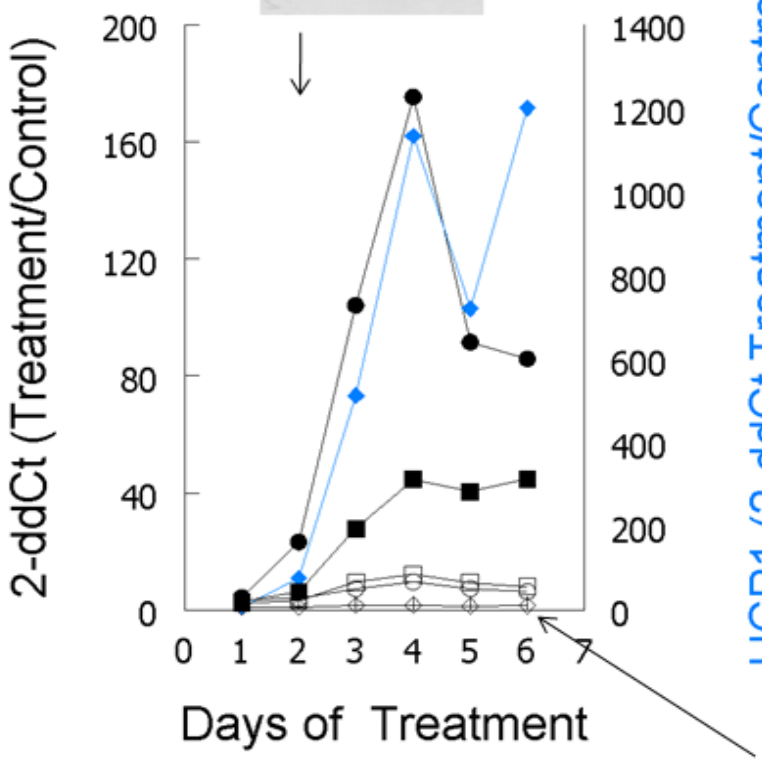
mTOR activity is measurably reduced after 2 days of treatment



OxPhos Western



MSDC-0160 μM
6 days of treatment



Note different scale for UCP1

- CPT1b
- UCP1
- PPARα
- PPARγ
- FABP4
- PGC-1α

Note: mitochondrial biogenesis with little effect on PGC1α

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Evaluate MSDC-0160 in PGC1α Null Mice

PGC1α null mice on C57BL/6 background [Burgess, et al ***J. Biol. Chem***., 2006; 281: 19000 – 19008]

- Effects on isolated progenitor cells *in vitro* WT and KO
  - Differentiation
  - UCP1 expression
  - Mitochondrial biogenesis

- Treatment of Wild Type and PGC1α knockout mice *in vivo* for 30 days with 30 mg/kg MSDC-0160. Tissues harvested and evaluated.
  - Intrascapular brown fat
  - Perirenal fat
  - Epididymal fat

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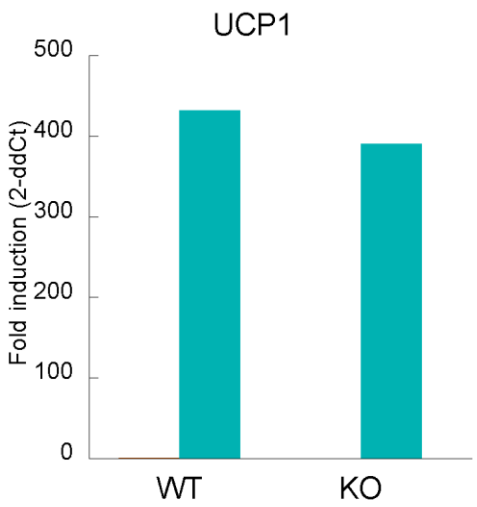
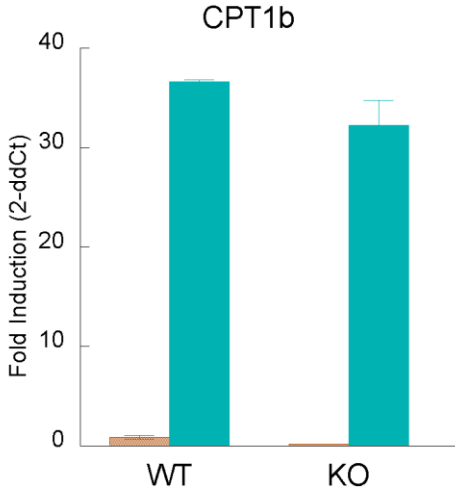
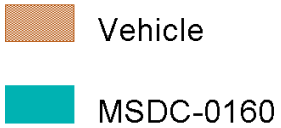
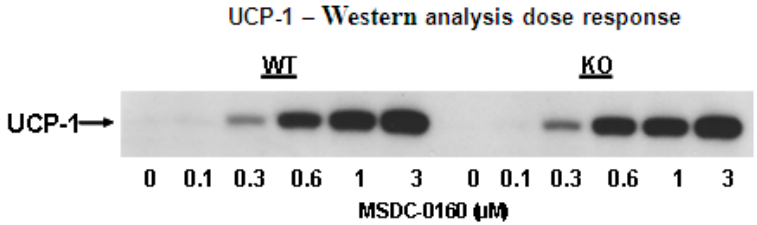
MSDC-0160 Effects *In Vitro* Are Independent of PGC-1α, Major PPARγ Coactivator (BAT precursors)



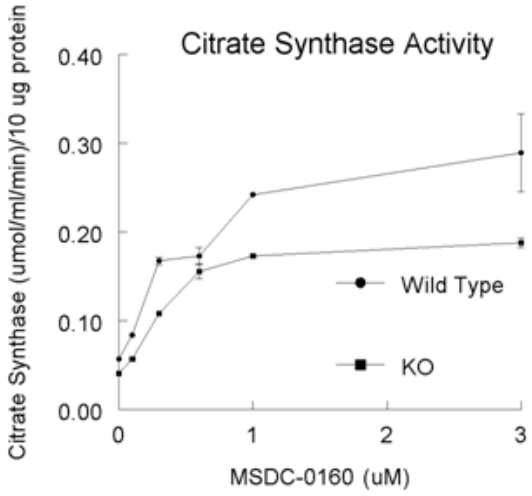


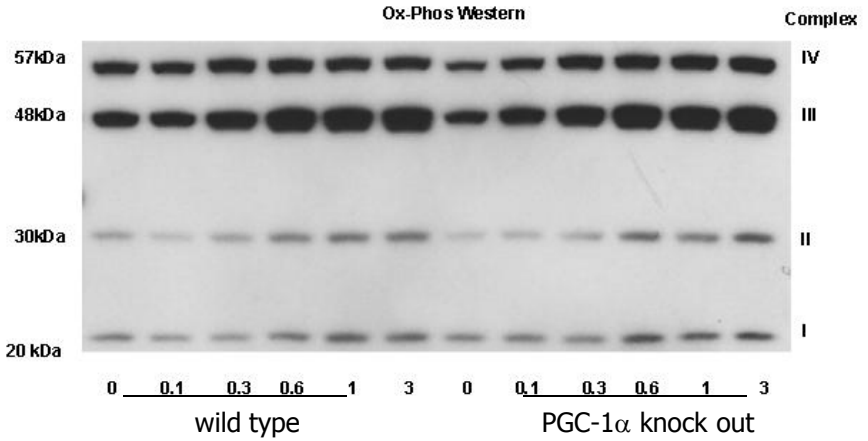


WT= wild type
KO= PGC-1α knock out



Mitochondrial biogenesis

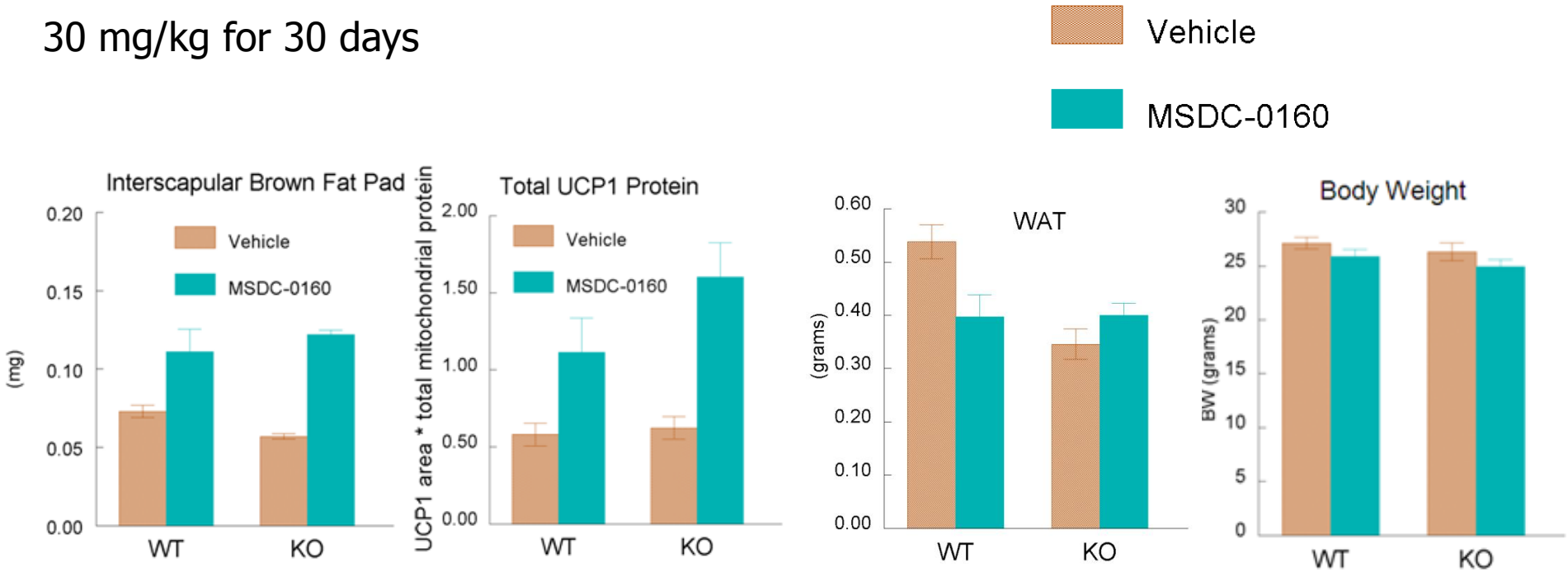




- The effects of MSDC-0160 are independent of PGC-1α
- BAT phenotype, mitochondrial biogenesis , FFA oxidation are favored

MSDC-0160 in WT and PGC-1α KO Mice *In Vivo*

30 mg/kg for 30 days



- MSDC-0160 increases functional brown fat in WT and PGC-1α KO mice
- In contrast epididymal fat (white adipose tissue) is reduced in mass
- Body weight tends to be reduced

WT= wild type
KO= PGC-1α knock out

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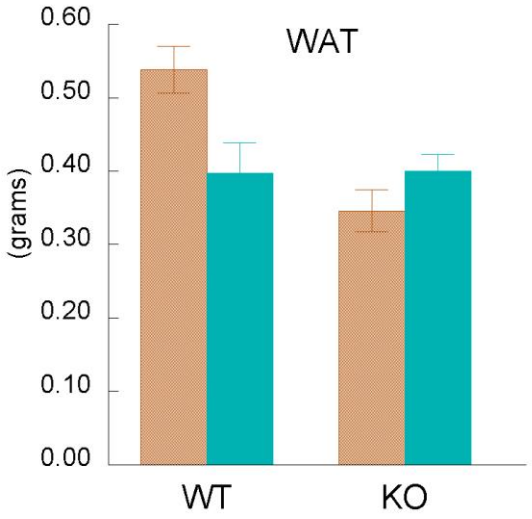
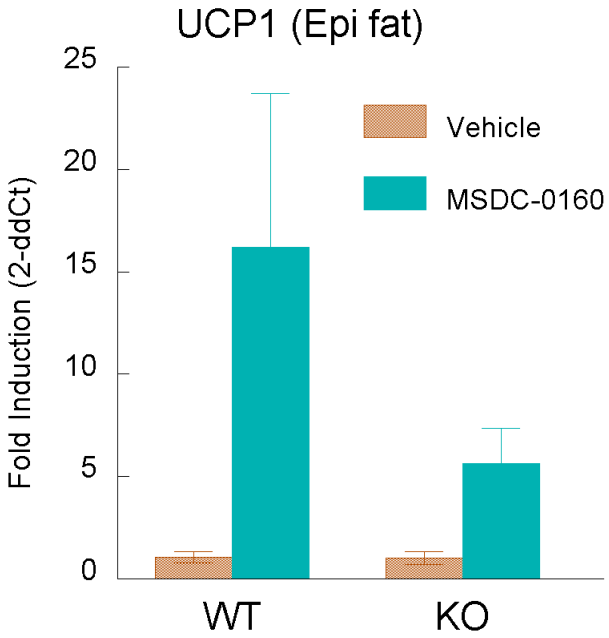
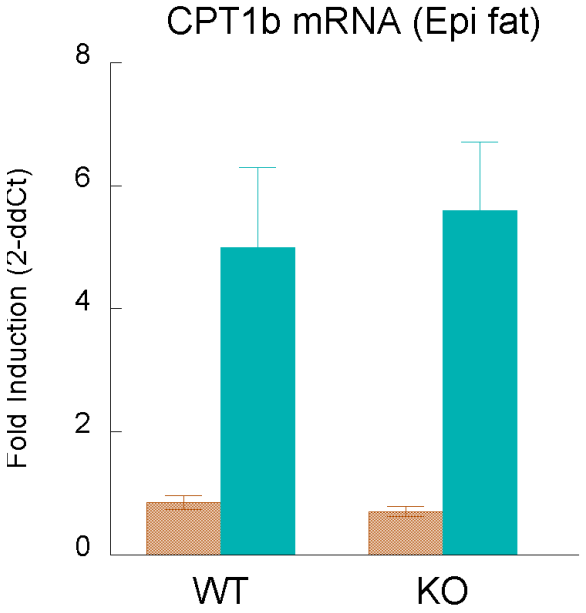
Epididymal Adipose Pad treatment of C57 mice in vivo

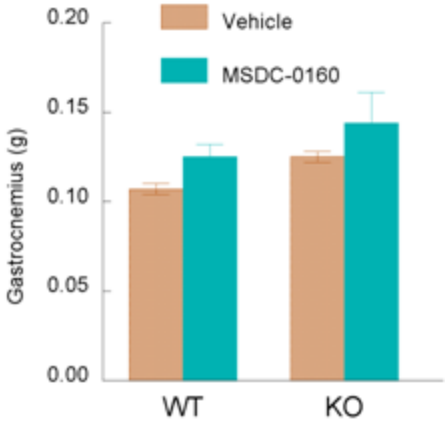
30 mg/kg for 30 days







muscle



- Unlike BAT, MSDC-0160 *decreased* the epi fat pad in WT (not KO) mice
- Increase in UCP1 and CPT1b in both WT and KO
- Dissection of gastrocnemius indicated that like BAT it was increased in size.

WT= wild type
KO= PGC-1α knock out

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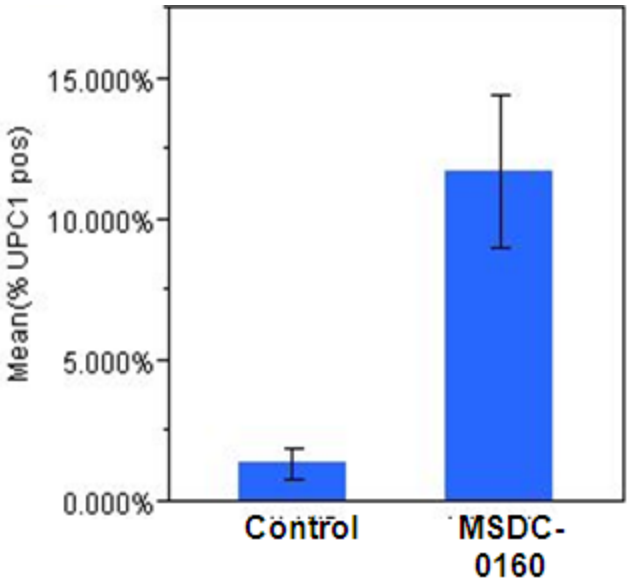
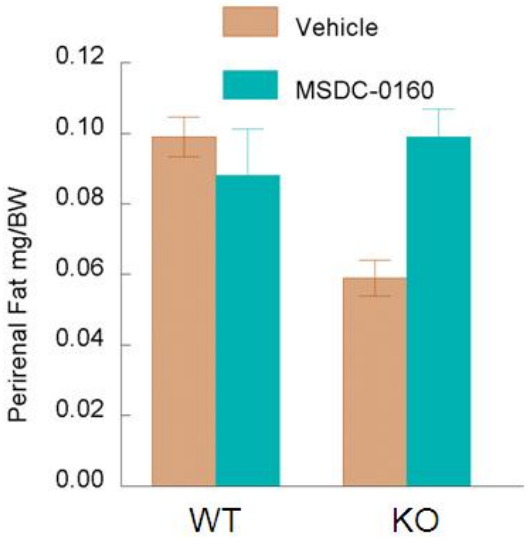
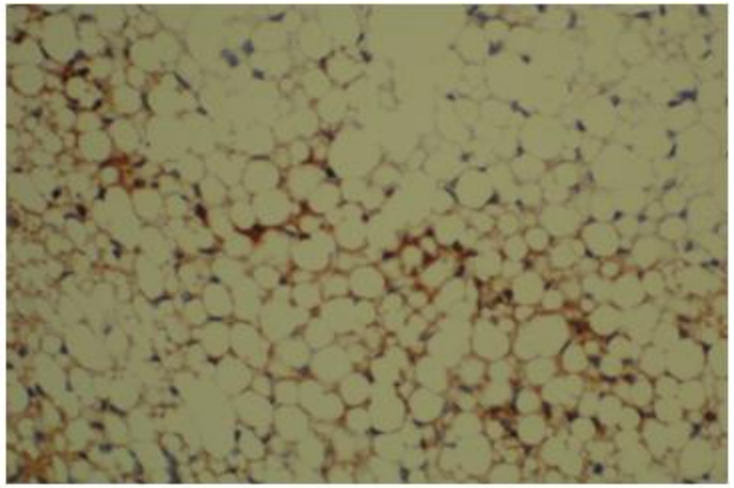
Increase in Perirenal UCP1 Expression in WT Mice (mixed response in PGC1α KO mice)



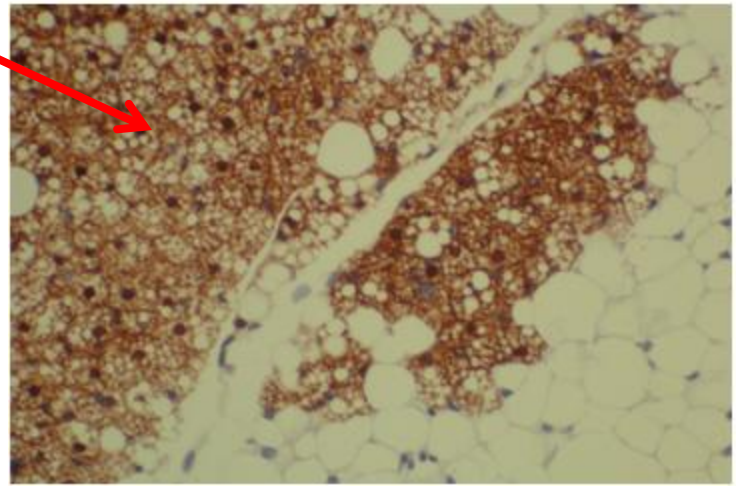


Control



Browning in perirenal adipose

MSDC-0160



Response blocked in PGC1α KO

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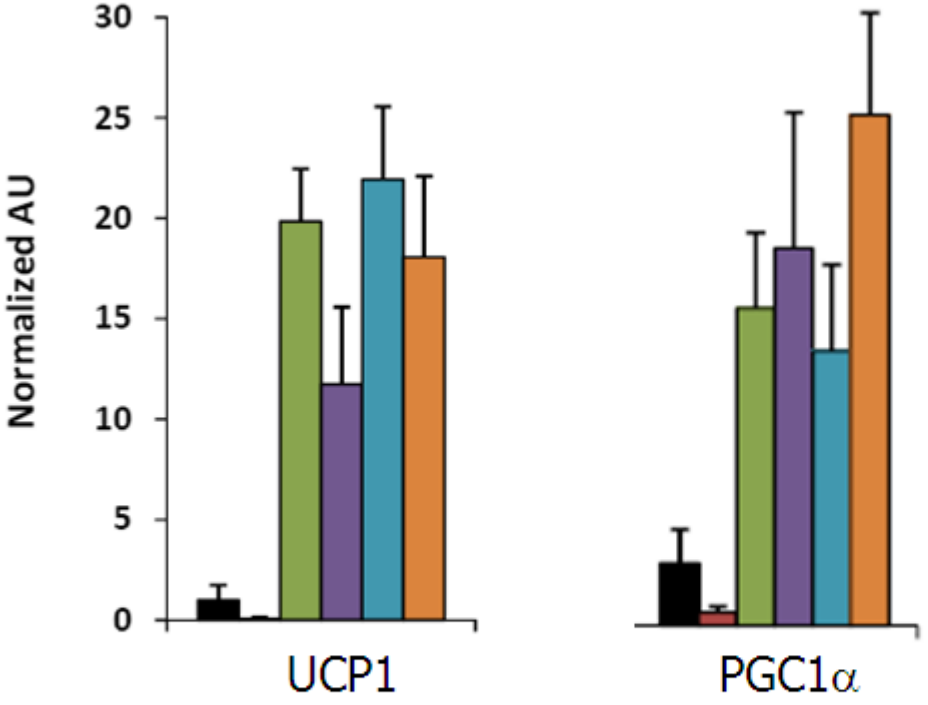
PPARγ-sparing TZDs Cause Adipocyte 'Browning' in ob/ob Mice As Well

Ob/ob mice treated for 4 weeks - Epididymal fat pad



While some drug effects may not be dependent on PGC1α, overall effects include these pathways

See also poster **1728P** Brian Finck, *et al* . Insulin Resistance in ob/ob Mice Is Ameliorated by Thiazolidinediones That Do Not Activate PPARγ

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Effects Independent of PPARγ

Adipose-specific peroxisome proliferator-activated receptor γ knockout causes insulin resistance in fat and liver but not in muscle

Weimin He*†, Yaacov Barak†‡§, Andrea Hevener*†, Peter Olson§¶, Debbie Liao§, Jamie Le*, Michael Nelson§, Estelita Ong§, Jerrold M. Olefsky*‖, and Ronald M. Evans§‖**

Departments of *Medicine and ¶Biology, University of California at San Diego, La Jolla, CA 92093; ‖The Jackson Laboratory, Bar Harbor, ME 04609; and §Gene Expression Laboratory and **Howard Hughes Medical Institute, The Salk Institute, La Jolla, CA 92037

Contributed by Ronald M. Evans, October 15712–15717 | PNAS | December 23, 2003 | vol. 100 | no. 26

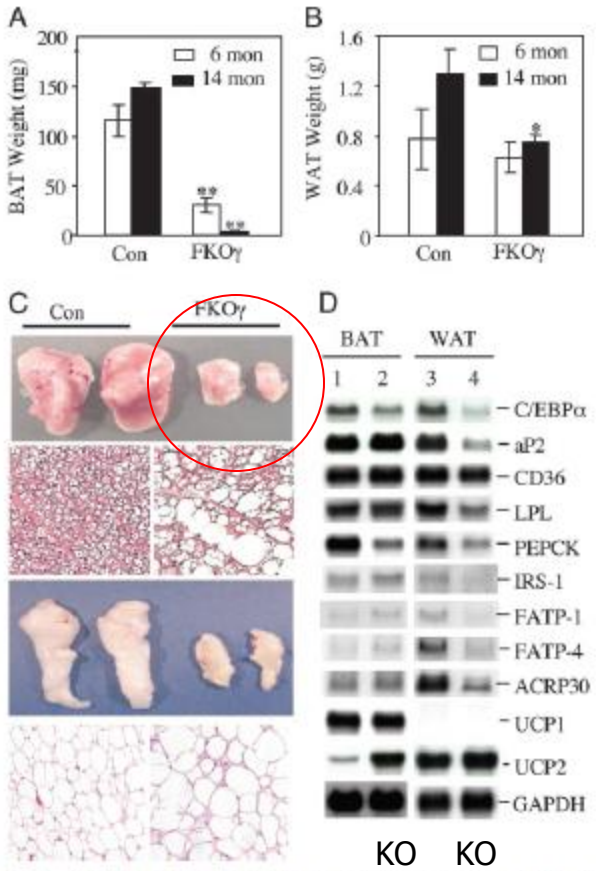

Fig. 2. Deletion of PPARγ in fat leads to progressive lipodystrophy. BAT (*A*) and WAT (*B*) weight changes in 6- and 14-month-old Con and FKOγ mice. Values are the mean ± SEM ($n = 10$). (*C*) Gross morphology and histology of BAT (top two panels) and WAT (bottom two panels) from 6-month-old Con and FKOγ mice. (*D*) Northern blot analysis of adipocyte genes in BAT and WAT in Con (lanes 1 and 3) and FKOγ (lanes 2 and 4) mice. Total RNA from each group ($n = 5$) was pooled, and 10 μg of RNA per group was analyzed.

- **Similar number of progenitor cells from WT and KO pads**
- **Full differentiation is arrested in these mice**
- **However, responses to MSDC-0160 are the same in progenitor cells from WT and KO**

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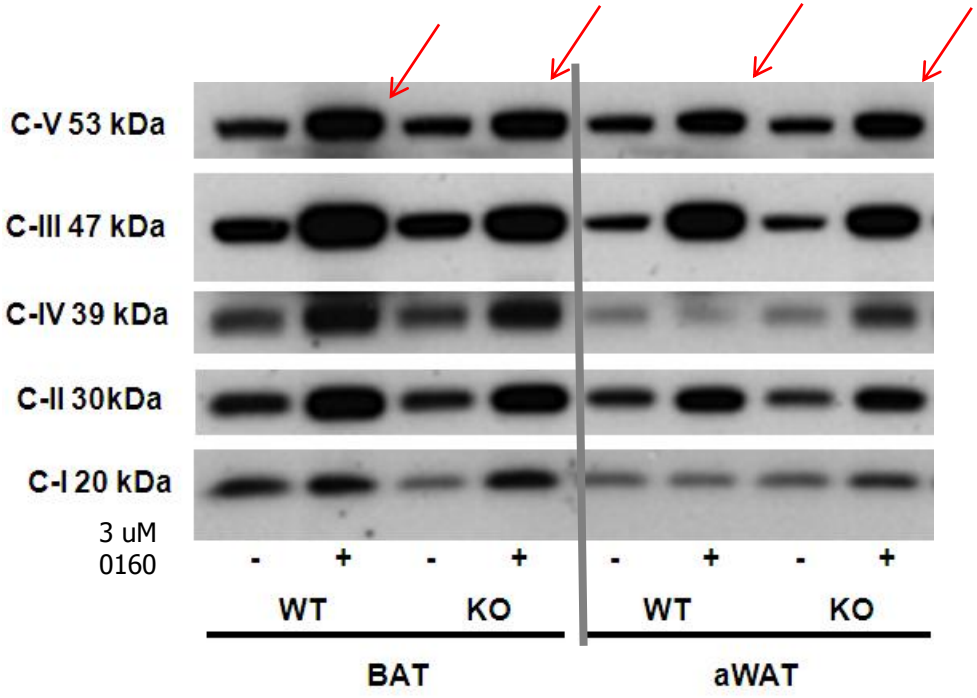
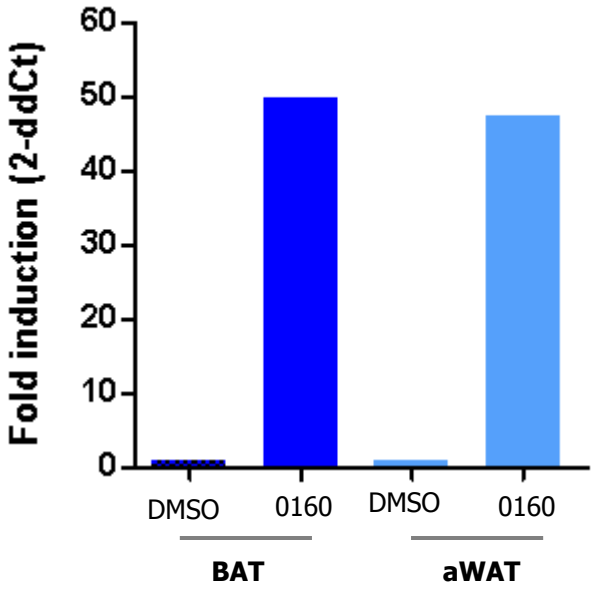
Some Effects Persist in PPARγ-null Cells

Increased Mitochondrial Biogenesis
Western Blot



Increased UCP1 message
Knockout



NOTE:

- Differentiation of BAT cells is attenuated in the PPARγ Knockout
- Increase in UCP1 message is 100 fold reduced and protein does not increase
- However, drug induced actions still occur similarly in both the WT and KO

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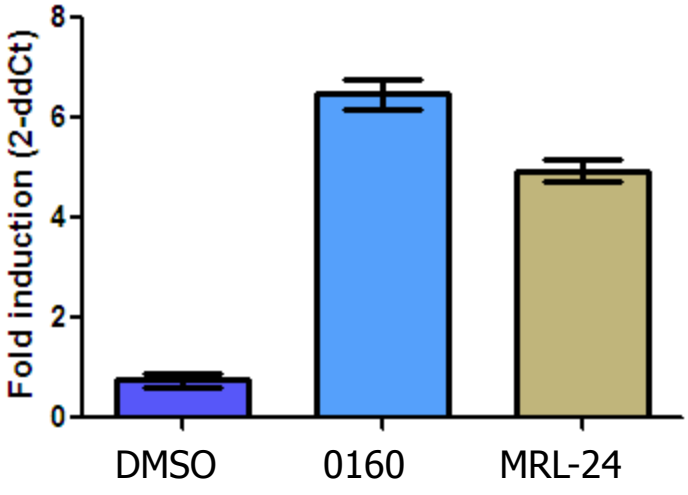
Effects in PPARγ-null Cells

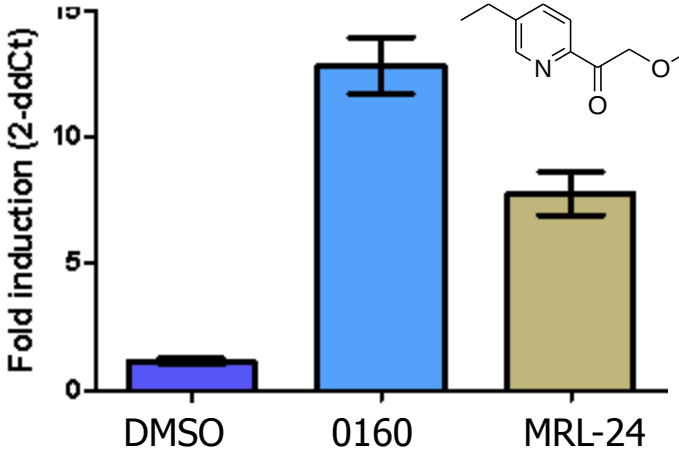
(compounds at 1 μM for 6 days) TZD and non-TZD compounds

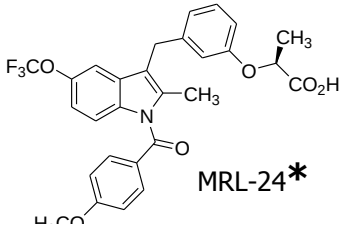
aP2

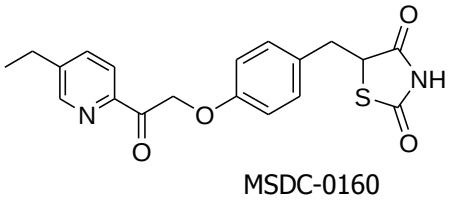
Knockout



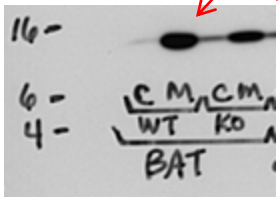
Wild Type







- MSDC-0160 and MRL-24 have similar effects in the knockout as in the wild type.
- Both message and protein are increased independent of PPARγ or differentiation.



C= DMSO
M= 0160

aP2 Western

*

Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPARgamma by Cdk5.
JH Choi, AS Banks, JL Estall, S Kajimura, P Bostrom, D Laznik, JL Ruas, MJ Chalmers, TM Kamenecka, M Bluher, PR Griffin, and BM Spiegelman
Nature 2010; 466(7305): 451-6.

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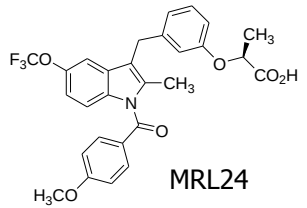
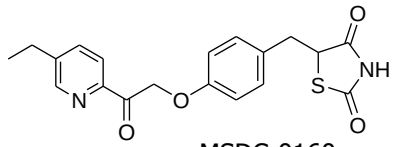
Effects in PPARγ-null Cells

(compounds at 1 μM for 6 days) TZD and non-TZD compounds

Adiponectin

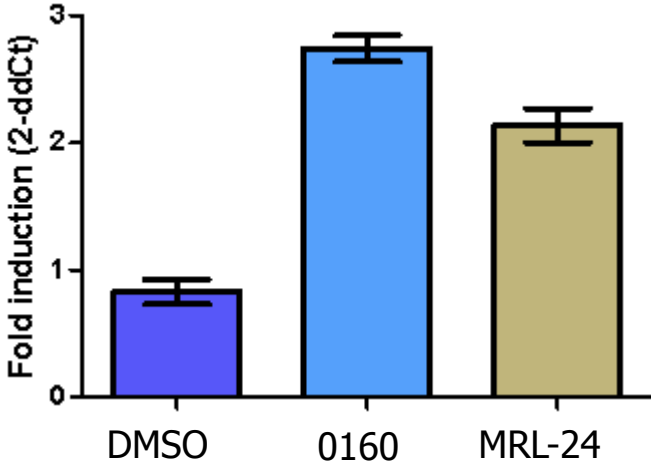


MRL24

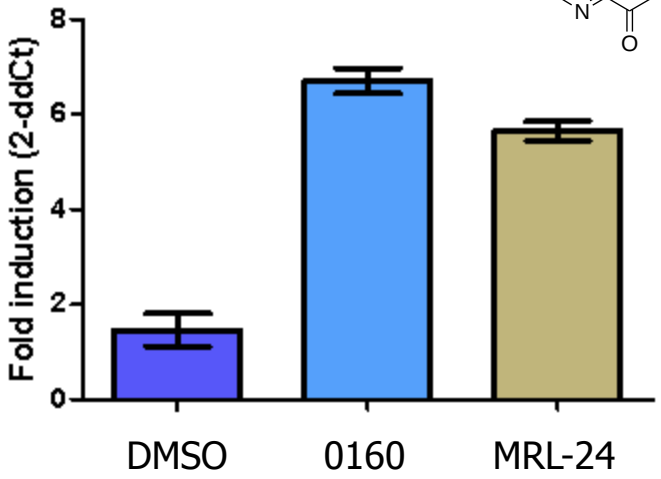


MSDC-0160

Knockout



Wild Type



- MSDC-0160 and MRL-24 have similar effects in the knockout as in the wild type.
- Drug-induced increase independent of PPARγ.

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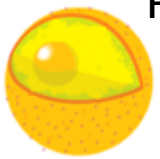
Mechanism of Action for Insulin Sensitizers

Old

Troglitazone; Rosiglitazone; Pioglitazone

Original TZDs



PPAR-Driven Gene Changes

Fat Sequestered
Increased Insulin Action
Fluid Retention
Weight Gain

New

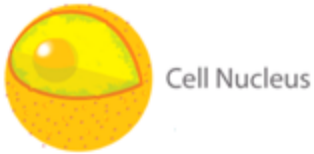
MSDC

Mito Target of TZDs (mTOT)

MSDC-0160
MSDC-0602

(Phase 2 clinical trials)

Nutrient signaling (Wnt pathway)



Nuclear Regulatory Factors

Increased Brown Fat
Preservation of β-cells

Improved Insulin Action
Improved Lipid Profiles

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Conclusions

- Presentations from this Symposium demonstrate the potential for treating diabetes by modifications in adipose or brown adipose tissues.
  - SWARBRICK, et. al. Intra-Abdominal Transplantation of Subcutaneous Adipose Tissue Ameliorates High-Fat Diet-Induced Glucose Intolerance and Adiposity in Mice.
  - STANFORD, et al Transplantation of Brown Adipose Tissue Exerts Beneficial Effects on Glucose Homeostasis
- These results indicate that a selective mitochondrial action of small molecules currently in clinical trials can augment brown adipose tissue in the intrascapular pad and cause "browning" in other adipose stores.
- The potential benefit of this mechanism is being evaluated in clinical trials.

Extra Slides Regarding Mechanism

See also poster **1603P** Bill McDonald, *et al .* Novel Insulin Sensitizers Enhance Brown Adipose Cell Differentiation by Modulation of the Wnt Signaling Pathway

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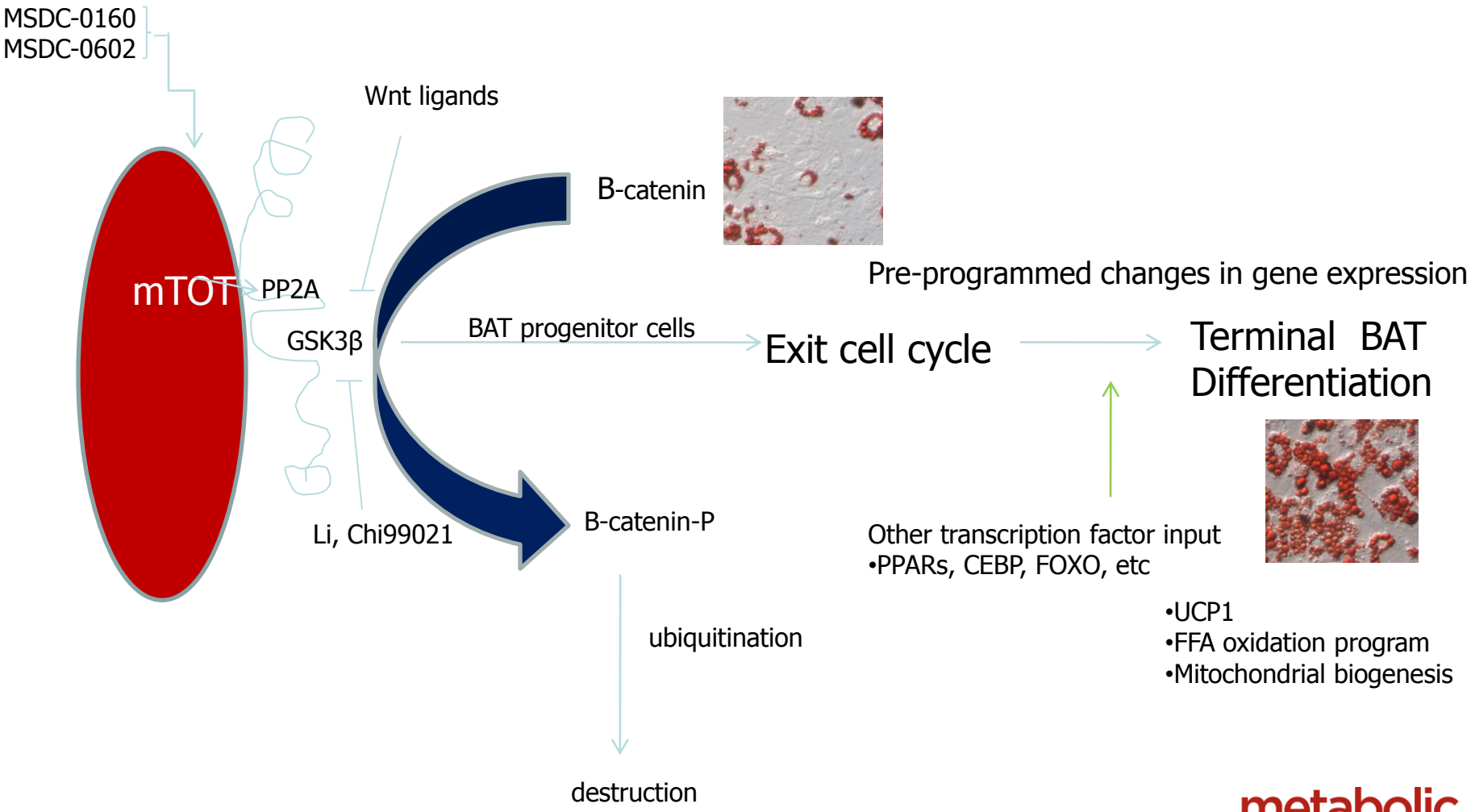
Summary of Current Knowledge

- Not blocked by PPARγ antagonists
- Occurs in PGC1α and PPARγ knockouts
- Involves a change in nutrient sensing pathways
- Earliest effect on phosphatase activity
- *Involves modification of Wnt signaling pathway*

- *Importance of mTOT in this signaling is currently under intense investigation*

1729-P White, et al. A Mitochondrial Target of Pioglitazone Acutely Regulates Mitochondrial Respiratory Function

Mitochondrial Molecular Switch for Terminal Differentiation of BAT: Attenuation of Wnt Signaling by the PPARγ-sparing TZDs



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Over-Activation of Wnt Pathways = Diabetes Risk

- **Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes.** SF Grant, *et al*. Nat Genet, 2006; 38(3): 320-3.

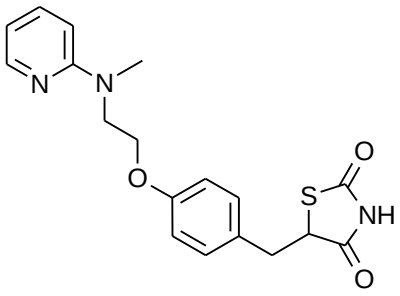
- **Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes.** Valeriya Lyssenko, *et al*. J Clin Invest. 2007;117(8):2155–2163 (overexpression reduces insulin secretion)

- **Association of the gene encoding wingless-type mammary tumor virus integration-site family member 5B (WNT5B) with type 2 diabetes.** A Kanazawa, *et al*. Am J Hum Genet, Nov 2004; 75(5): 832-43.

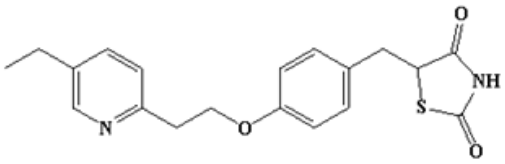
- **The effect of WNT5B IVS3C>G on the susceptibility to type 2 diabetes in UK Caucasian subjects.** KD Salpea, *et al*. Nutr Metab Cardiovasc Dis, Feb 2009; 19(2): 140-5*.

***CONCLUSION**: Variation in WNT5B predisposes to T2D in the absence of obesity. The increase in risk conferred by the presence of both WNT5B and TCF7L2 variants strengthens the role of Wnt signaling in T2D.

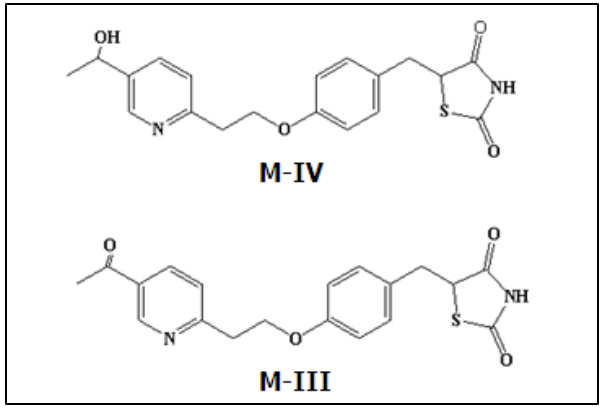
Pioglitazone and Its Major Metabolites; Other Compounds

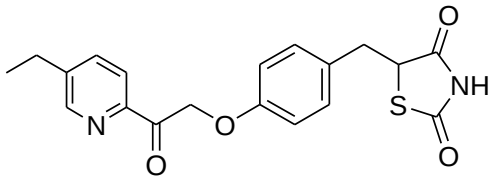


Rosiglitazone

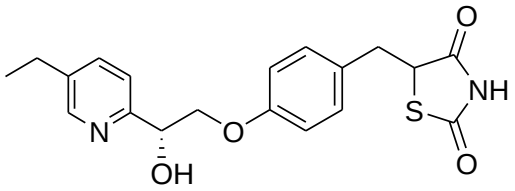


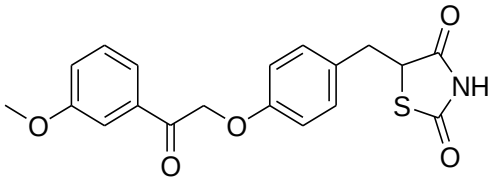
Pioglitazone



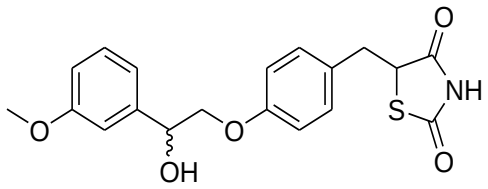


MSDC-0160





MSDC-0602



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