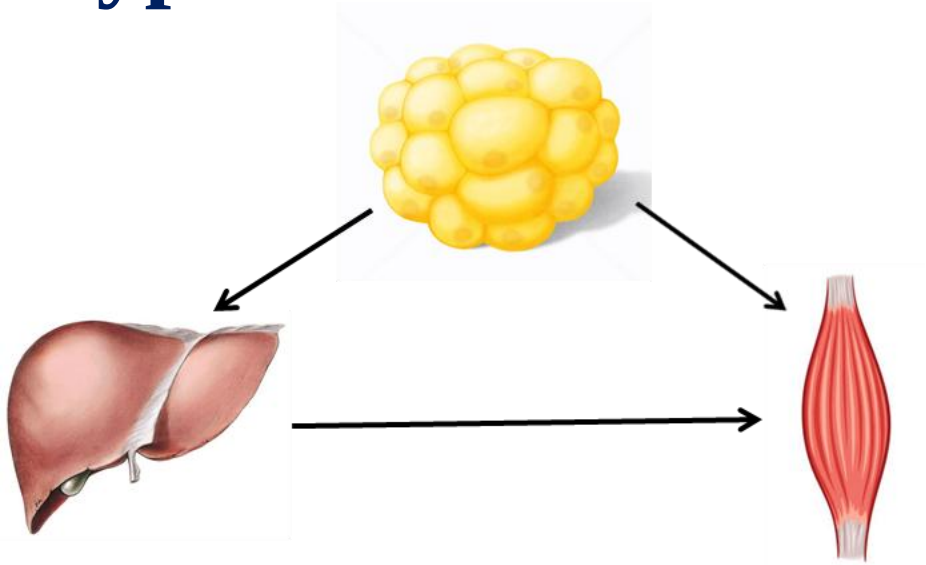
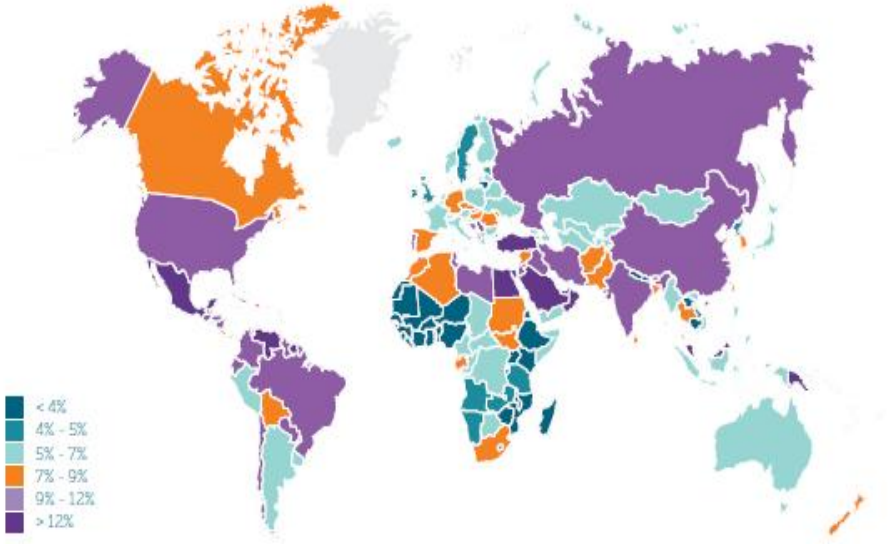
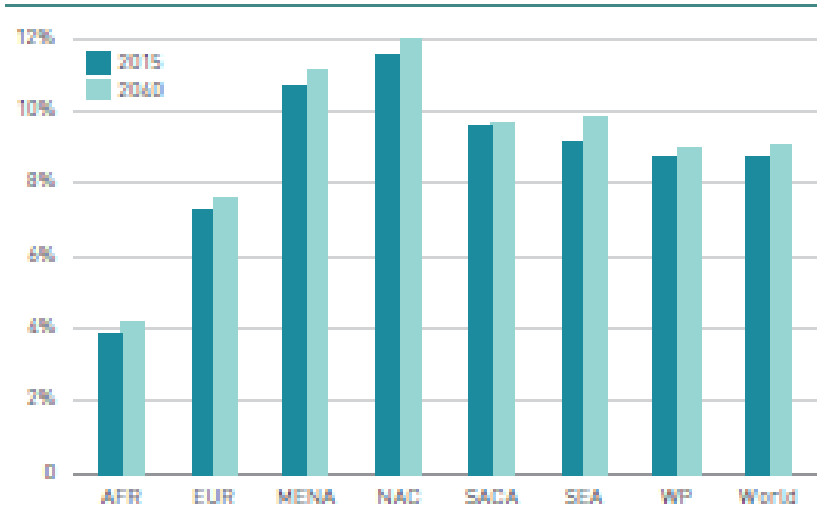
Effects of growth hormone secretagogue receptor agonist and antagonist in non-obese type 2 diabetic MKR mice



Rasha Mosa
(MBCHC, M.D, PhD candidate)
School of Biomedical Sciences
University of Queensland

Diabetes is on the rise worldwide

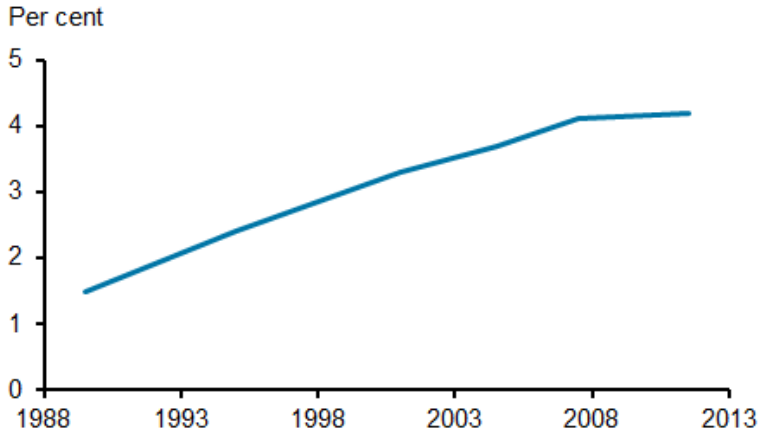




- **Global prevalence:**
  - 2015: 8.8% (7.2-11.4%) 415 million people
  - 2040: 10.4% (8.5-13.5%) 642 million people

Diabetes in Australia

- 280 Australians develop diabetes every day.
- Around 1.7 million Australians have diabetes.
- Total annual cost impact of diabetes in Australia estimated at $14.6 billion



AIHW
Authoritative information and statistics to promote better health and wellbeing

Diabetes in Egypt

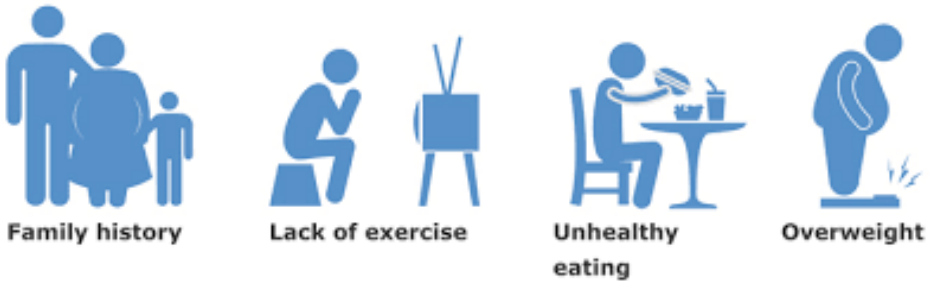
- There were over 7.8 million cases of diabetes in Egypt in 2015.
- Egypt have more people under the age of 60 with diabetes compared to the world average.

Rank	Country/territory	2015 Number of people with diabetes
1	China	109.6 million (99.6-133.4)
2	India	69.2 million (56.2-84.8)
3	United States of America	29.3 million (27.6-30.9)
4	Brazil	14.3 million (12.9-15.8)
5	Russian Federation	12.1 million (6.2-17.0)
6	Mexico	11.5 million (6.2-13.7)
7	Indonesia	10.0 million (8.7-10.9)
8	Egypt	7.8 million (3.8-9.0)
9	Japan	7.2 million (6.1-9.6)
10	Bangladesh	7.1 million (5.3-12.0)

Rank	Country/territory	2040 Number of people with diabetes
1	China	150.7 million (138.0-179.4)
2	India	123.5 million (99.1-150.3)
3	United States of America	35.1 million (33.0-37.2)
4	Brazil	23.3 million (21.0-25.9)
5	Mexico	20.6 million (11.4-24.7)
6	Indonesia	16.2 million (14.3-17.7)
7	Egypt	15.1 million (7.3-17.3)
8	Pakistan	14.4 million (10.6-20.4)
9	Bangladesh	13.6 million (10.7-24.6)
10	Russian Federation	12.4 million (6.4-17.1)

Type 2 diabetes

- Type 2 diabetes mellitus is a complex endocrine and metabolic disorder.
- The risk factors include interaction between several genetic and environmental factors.
- Overweight and obesity are major contributors to the development of insulin resistance and impaired glucose tolerance.



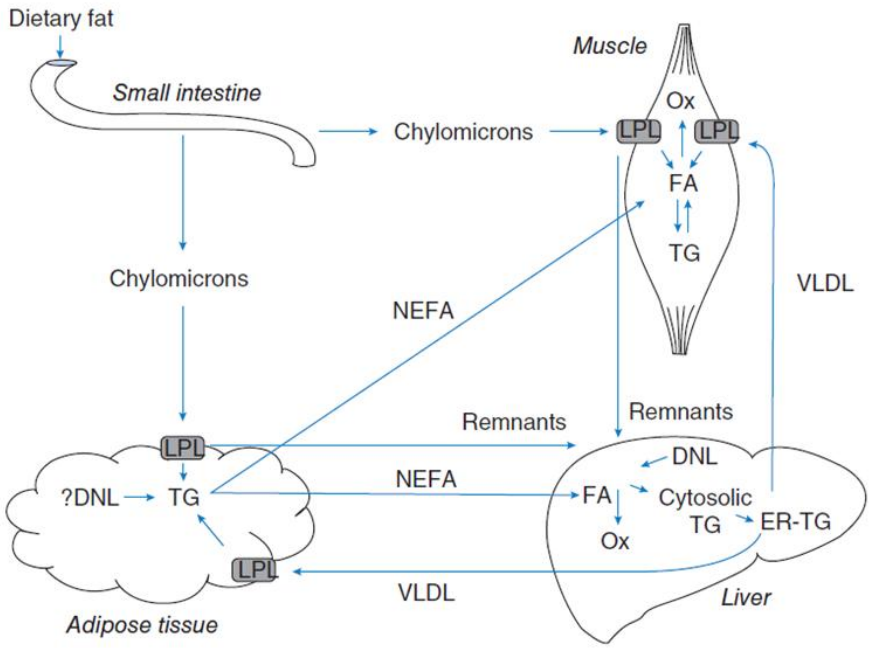
Normal weight yet metabolically obese



- WHO defined obesity as ***abnormal*** or ***excessive*** accumulation of fat that presents risk to health.
- Individuals who have high risk of insulin resistance have a tendency to accumulate of fat in the **visceral compartment** and in **non- adipose compartments** such as liver and muscle
- Waist circumference measurements provides a simple clinical tool to screen those who have expanded visceral fat compartments.

Adipose tissue and glucose homeostasis

- The adipose tissue, in spite of being a minor site for glucose uptake, play a major role in controlling overall glucose metabolism.
- Adipose tissue functioning as a **sink** for fatty acids to prevent an overload of lipid to other tissues.
- Adipose tissues secretes adipokines such as leptin and adiponectin.
- Transgenic ablation of white adipose tissue in mice leads to severe insulin resistance.



Adapted from Keith N. Frayn et al, 2006

Mechanism of ectopic fat deposition

- Failure to develop adequate subcutaneous fat (lipodystrophy).

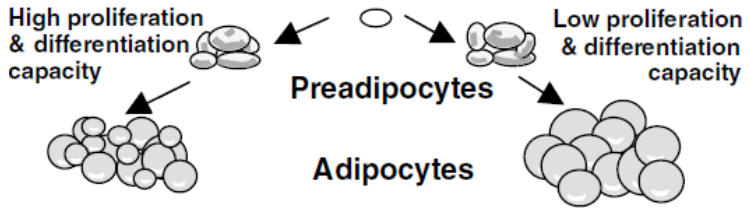


- Failure of new fat cell formation

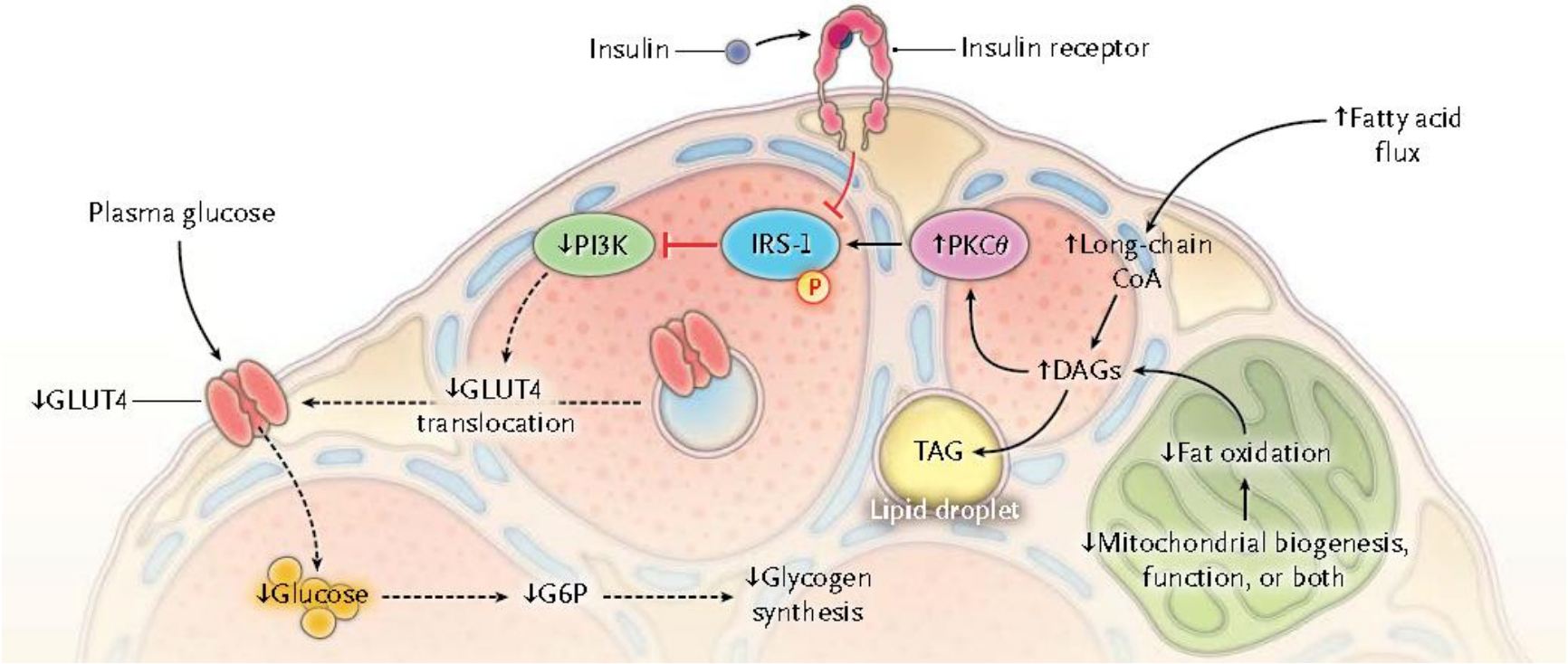
Small adipocytes have the ability to accumulate triglyceride than the larger adipocytes.

Large adipocytes are likely to secrete an entirely different pattern of hormones than small adipocytes

- Increase lipolysis of adipose tissue.

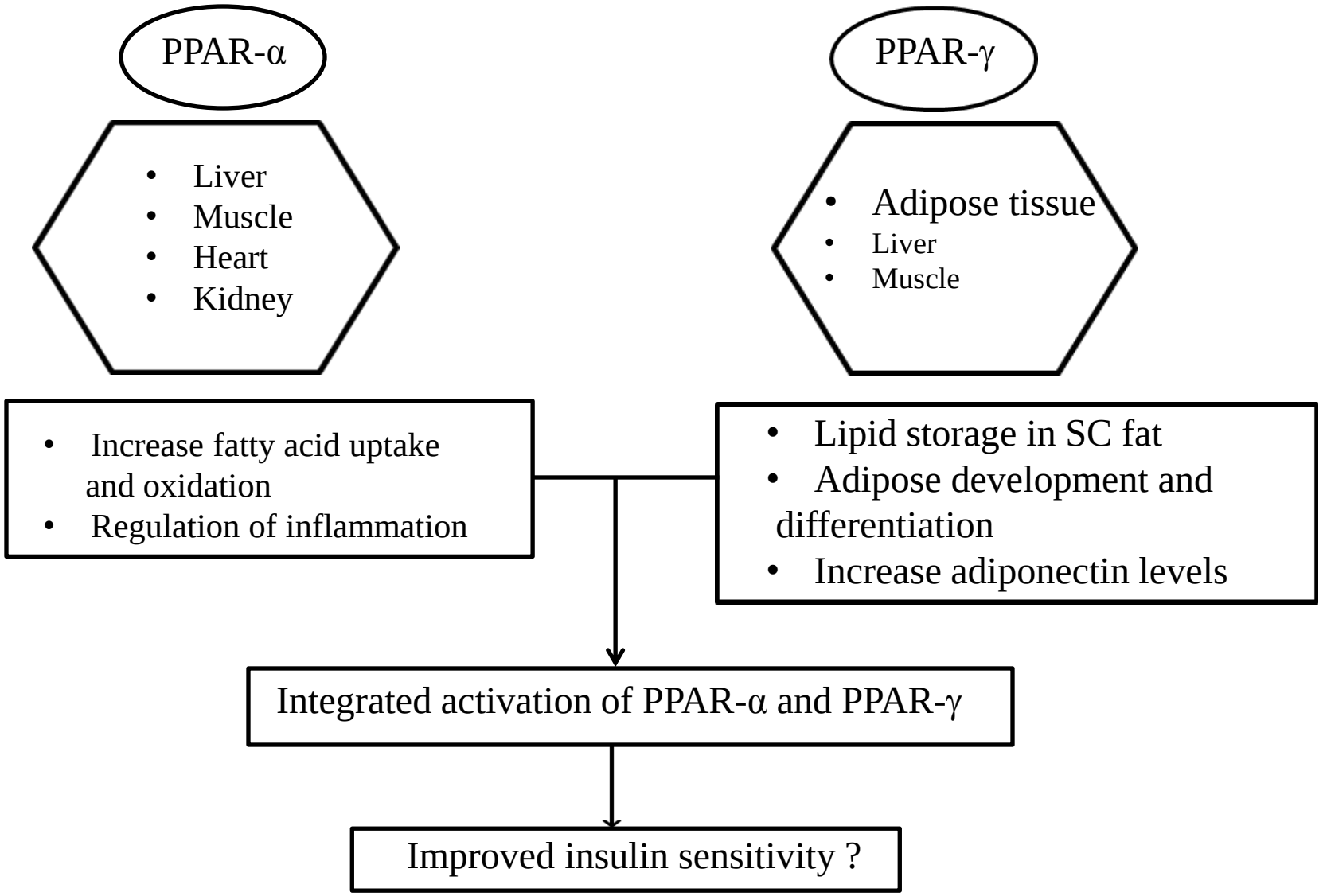


Impaired fat oxidation



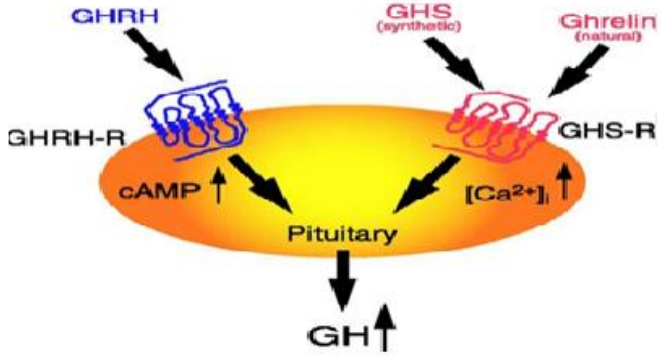
adapted from Gerald I. Shulman, 2014

Role of PPAR-α and γ in insulin sensitization



Growth hormone releasing peptides (GHRPs)

- The GHRPs are small synthetic peptides stimulate growth hormone release through binding to the GH secretagogue receptor 1a (GHS-R1a), in hypothalamus and pituitary and later recognized as the receptor for ghrelin.
- Ghrelin, an endogenous GHS induce food intake, adiposity, and body weight gain.



Hexarelin

- Hexarelin is one of GHS which has GH-independent effects on:
  - Protection against cardiac ischemia and impairment of vascular endothelium function
  - Orexigenic properties
  - Fat metabolism
  - Bone cell differentiation

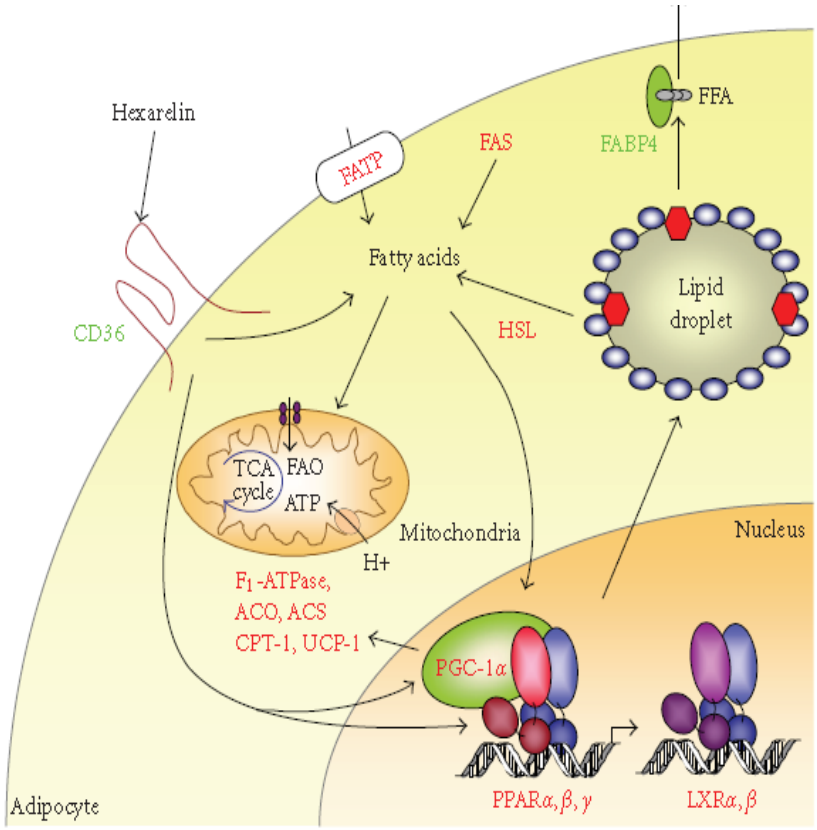
Hexarelin Signaling to PPARγ in Metabolic Diseases

Annie Demers,[1] Amélie Rodrigue-Way,[1,2] and André Tremblay[1,2,3]

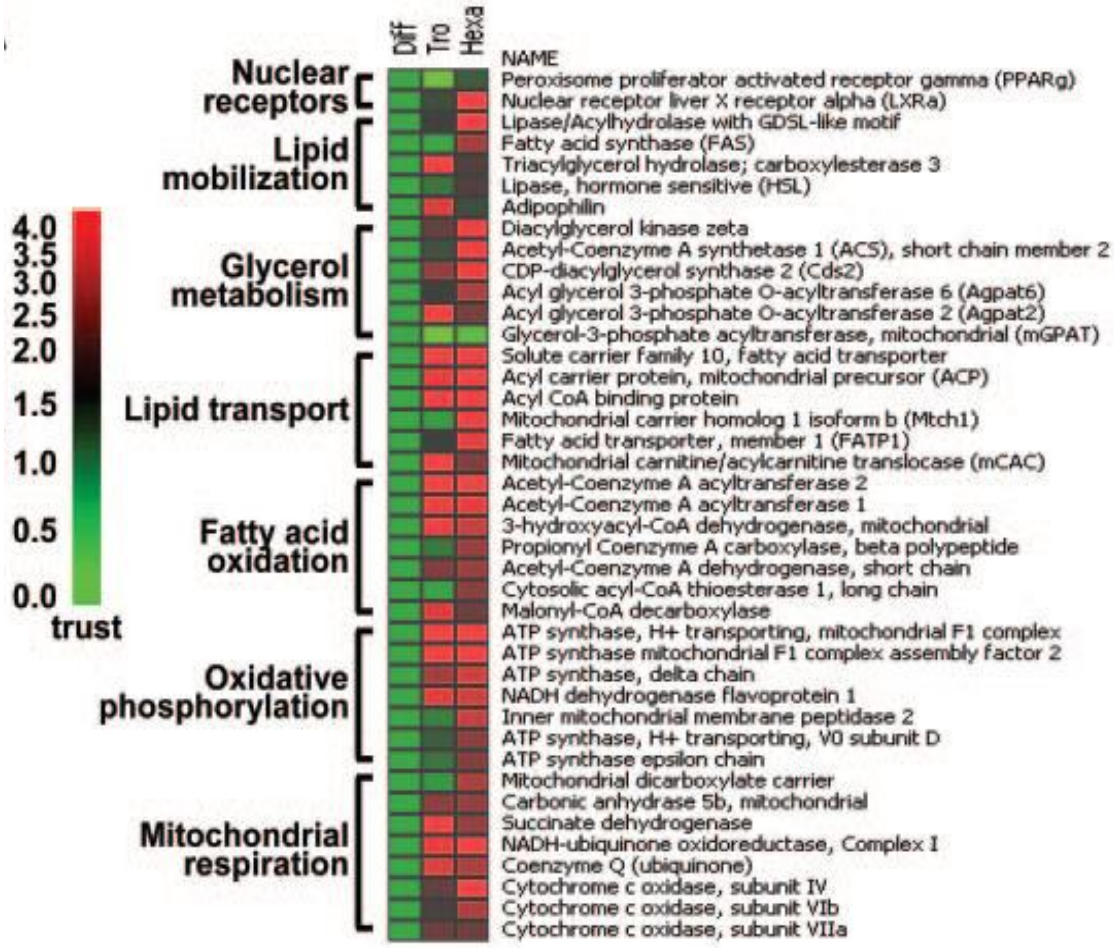
[1] *Research Center, Ste-Justine Hospital, University of Montreal, Montréal, PQ, Canada H3T 1C5*
[2] *Department of Biochemistry, University of Montreal, Montréal, PQ, Canada H3T 1J4*
[3] *Department of Obstetrics and Gynecology, University of Montreal, Montréal, PQ, Canada H3T 1C5*

Correspondence should be addressed to André Tremblay, andre.tremblay@recherche-ste-justine.qc.ca

- Hexarelin promote PPARγ activation to adipocytes and macrophages.
- Many genes of fatty acid oxidation and mitochondria morphology upregulated by hexarelin.
- Hexarelin can also promote PPARα and PPARβ/δ activation.



DNA microarray analysis of differentiated adipocytes treated with troglitazone, or hexarelin.



Hexarelin induced the expression of genes associated with fatty acid oxidation similar to Troglitazone treatment.

STOMACH

Antagonism of ghrelin receptor reduces food intake and body weight gain in mice

A Asakawa, A Inui, T Kaga, G Katsuura, M Fujimiya, M A Fujino, M Kasuga

- Peripherally administered GHS-R antagonists [D-Lys-3]-GHRP-6 and substance P decreased food intake in lean mice, in mice with diet induced obesity, and in ob/ob obese mice.
- Repeated administration of [D-Lys-3]-GHRP-6 decreased body weight gain and improved glycaemic control in ob/ob obese mice.

Mouse model-MKR mice

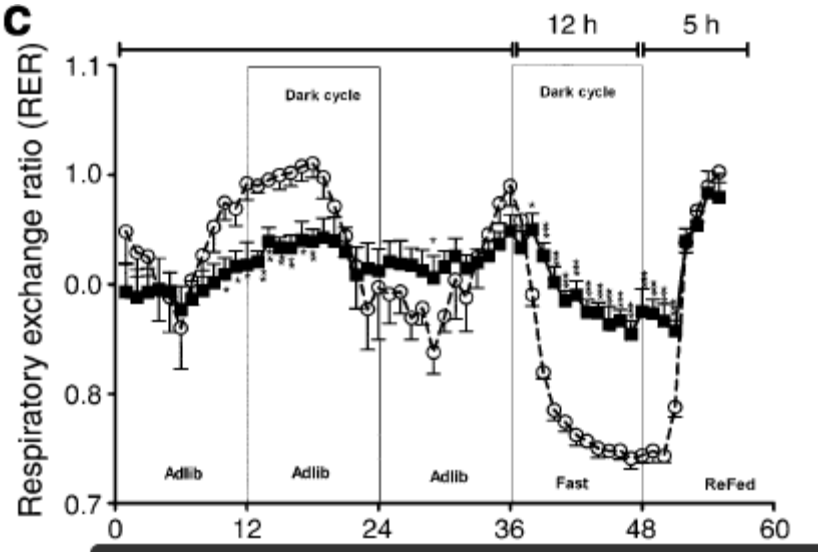
- Overexpress a dominant-negative IGF-I receptor in skeletal muscle.
- Insulin resistance characterized by:
  - An early-onset type 2 diabetes
  - Dyslipidemia
  - Hyperinsulinemia
  - Excessive lipid stores in muscle and liver
  - Beta-cell dysfunction
  - Impaired capacity to utilise fatty acids results in a compensatory increase in glucose disposal.

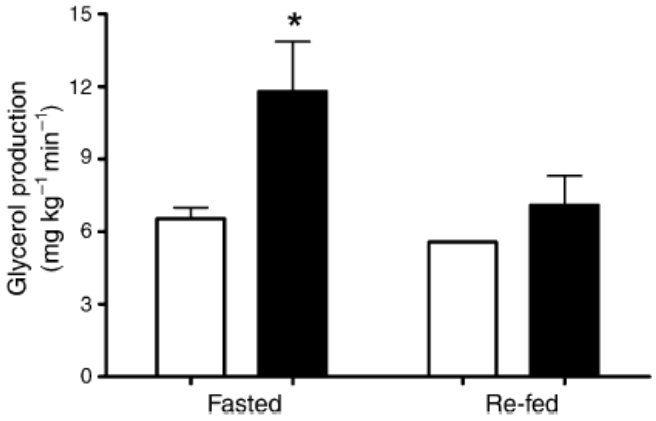
Diabetologia (2010) 53:2224–2232
DOI 10.1007/s00125-010-1827-4

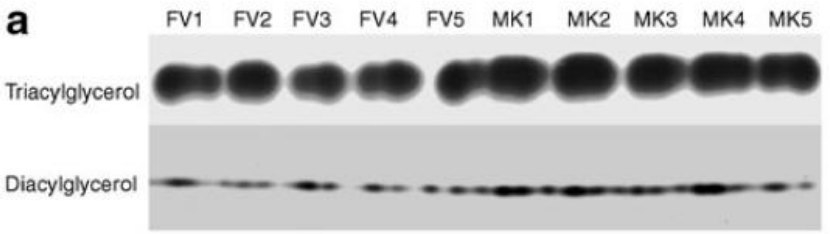
ARTICLE

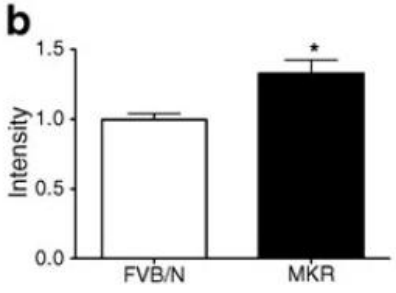
MKR mice have increased dynamic glucose disposal despite metabolic inflexibility, and hepatic and peripheral insulin insensitivity

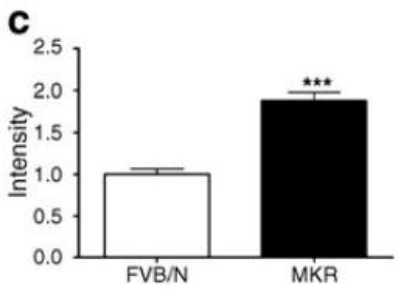
B. Vaitheesvaran · D. LeRoith · I. J. Kurland





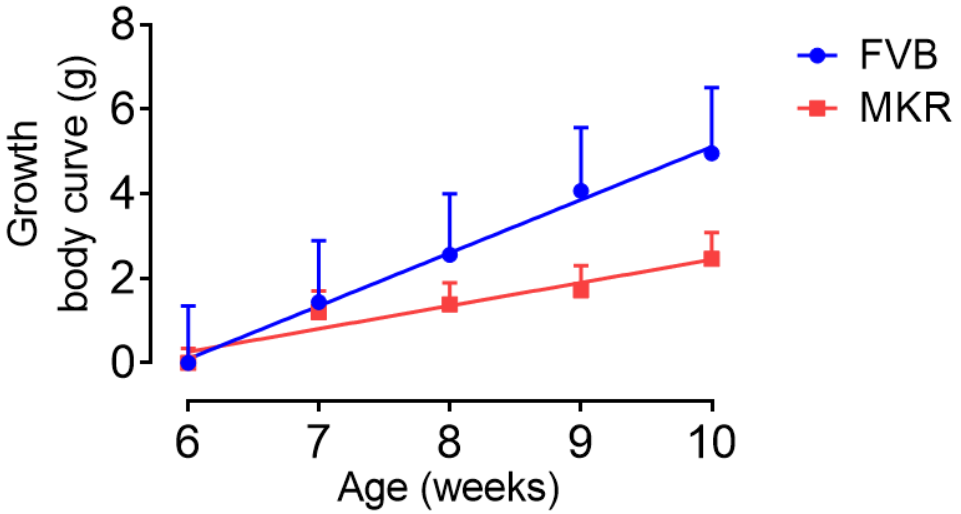


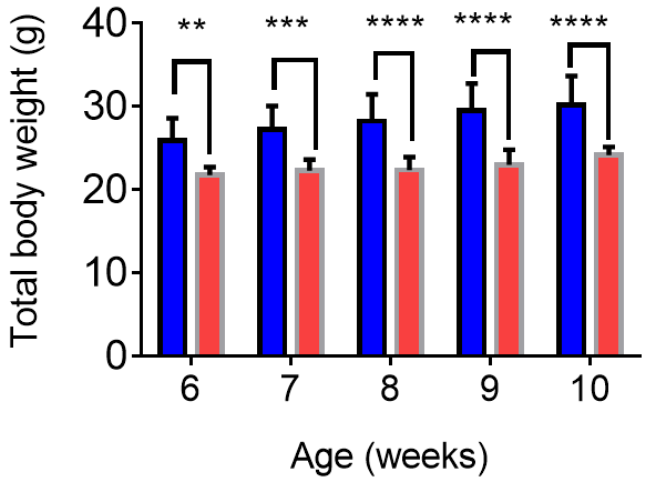




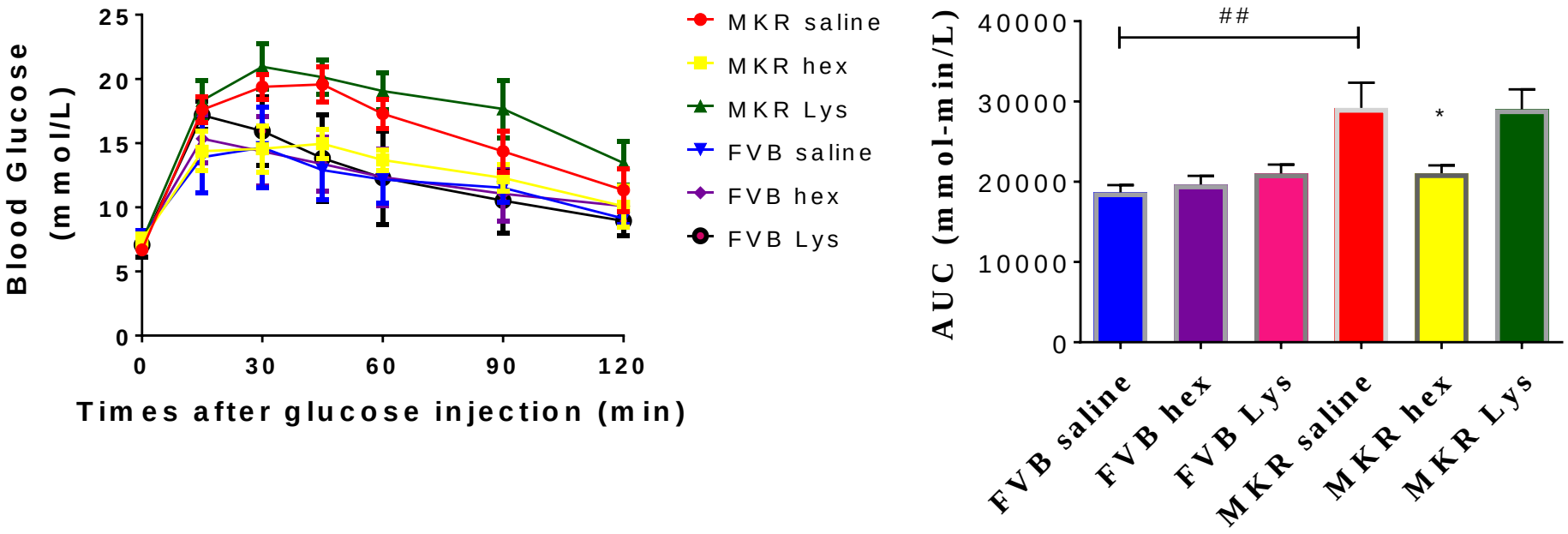
MKR mice have lower body weight compared to FVB mice (6-10 weeks)





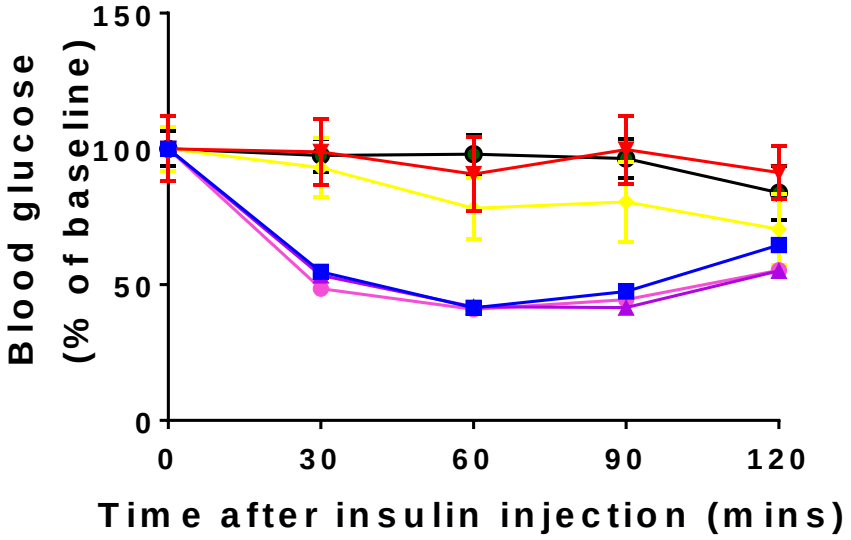


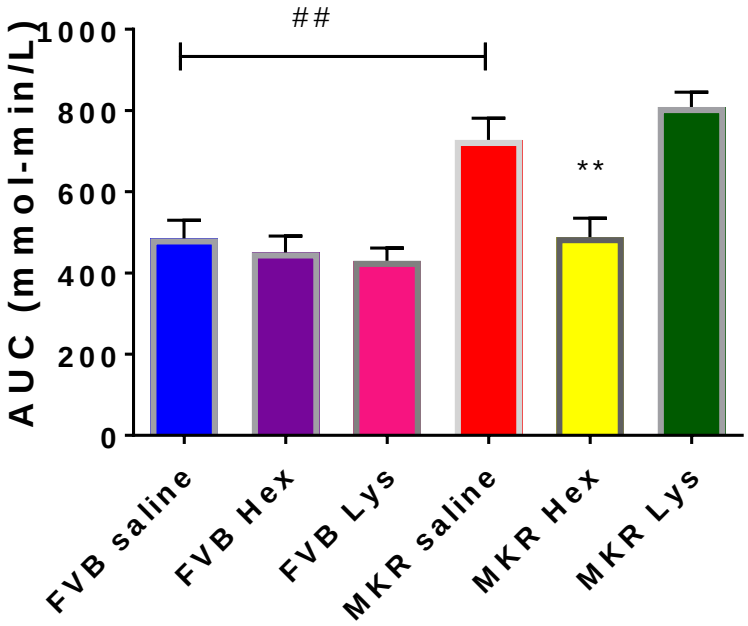
Hexarelin improved glucose tolerance test in MKR mice after 12 days of treatment



Intraperitoneal glucose tolerance test (2g/kg) performed on mice fasted for 6 hours. The results are presented as mean + SEM (n=5-6) * P<0.05 vs. the saline-treated MKR mice. ##, P <0.01 vs. the saline-treated WT mice

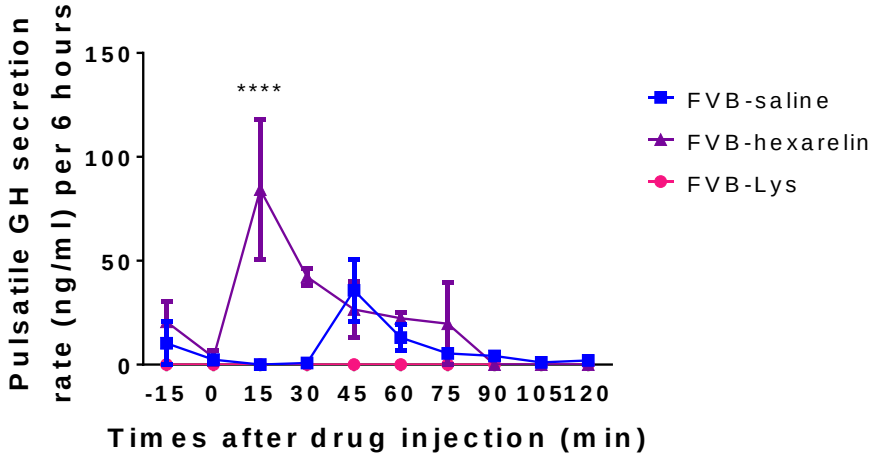
Hexarelin improved insulin tolerance test in MKR mice

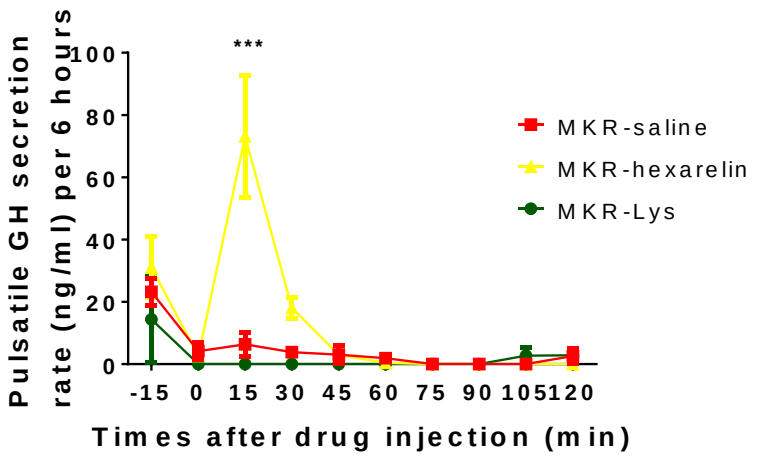


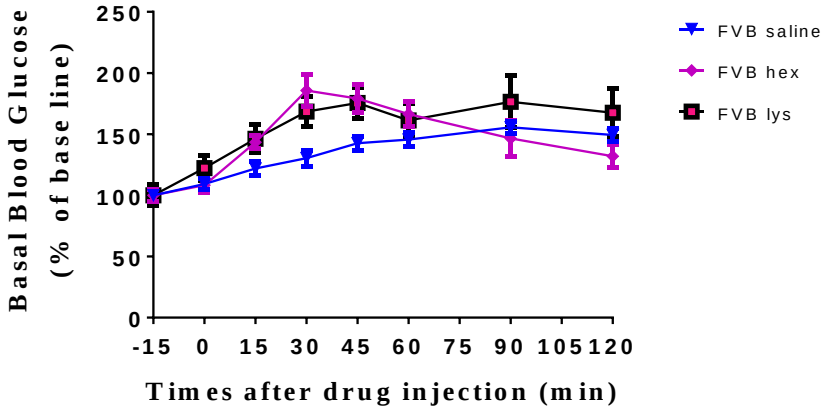


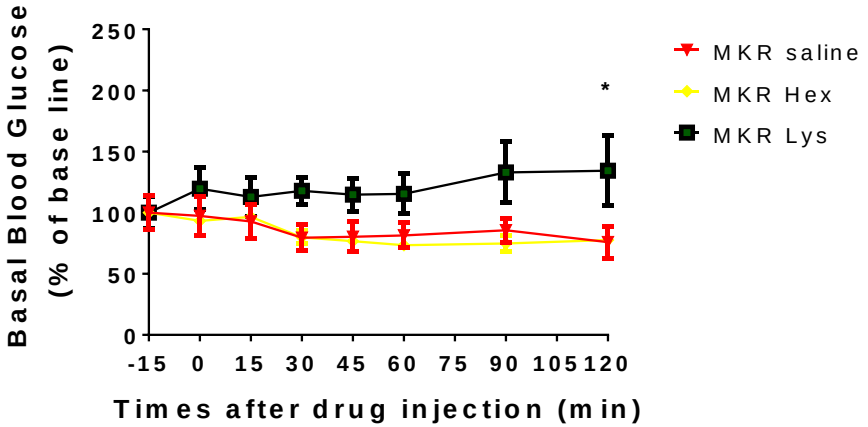
Intraperitoneal insulin tolerance test (0.75 U/kg) performed on mice fasted for 6 hours. The results are presented as mean + SEM (n=5-6) **P<0.01 vs. the saline-treated MKR mice. ##, P <0.01 vs. the saline-treated WT mice.

Hexarelin did increase GH secretion but not affect basal blood glucose in both MKR and WT mice



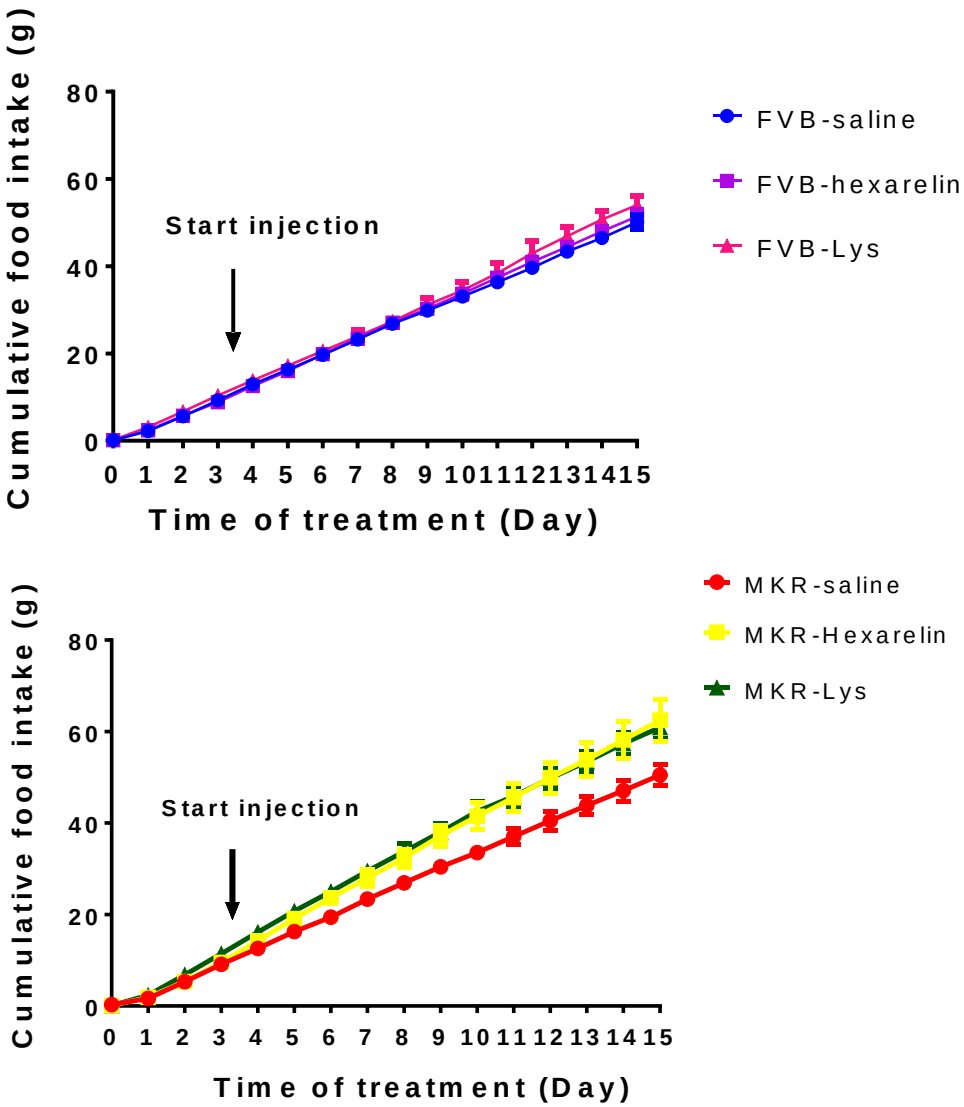


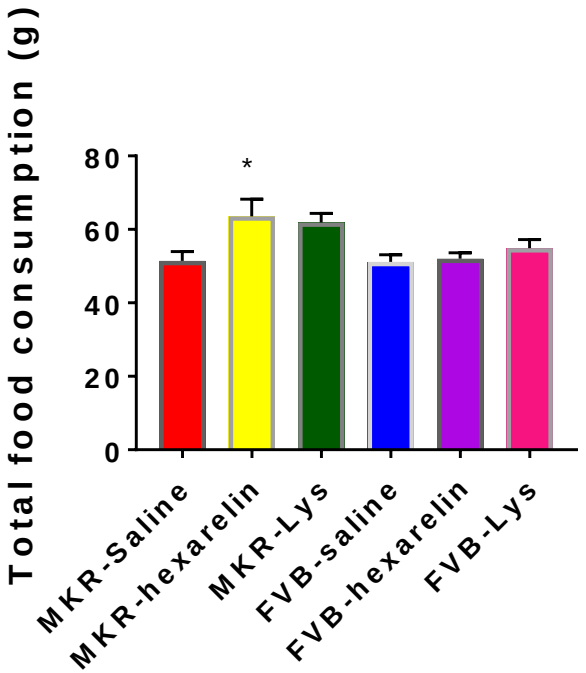




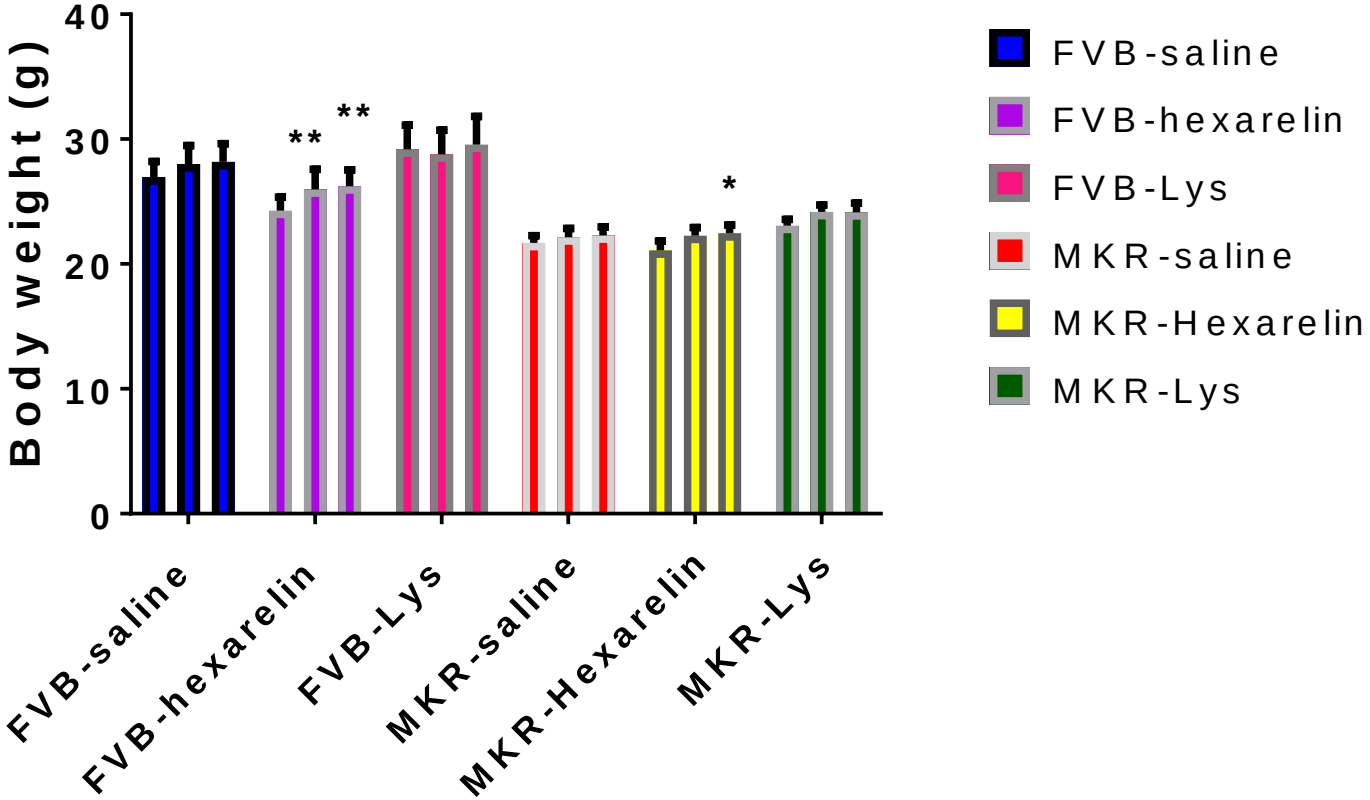
Effect of hexarelin and (D-LYS)-GHRP-6 on pulsatile GH secretion profiles and basal blood glucose in MKR mice (A, C) and corresponding wild type (B, D). Samples were collected for 2 h at 15 min intervals, after drug injection.(n=3-4). * P<0.05, .***P<0.001, ****P <.0.0001 vs. the saline-treated MKR mice.

Effect of treatment on food intake



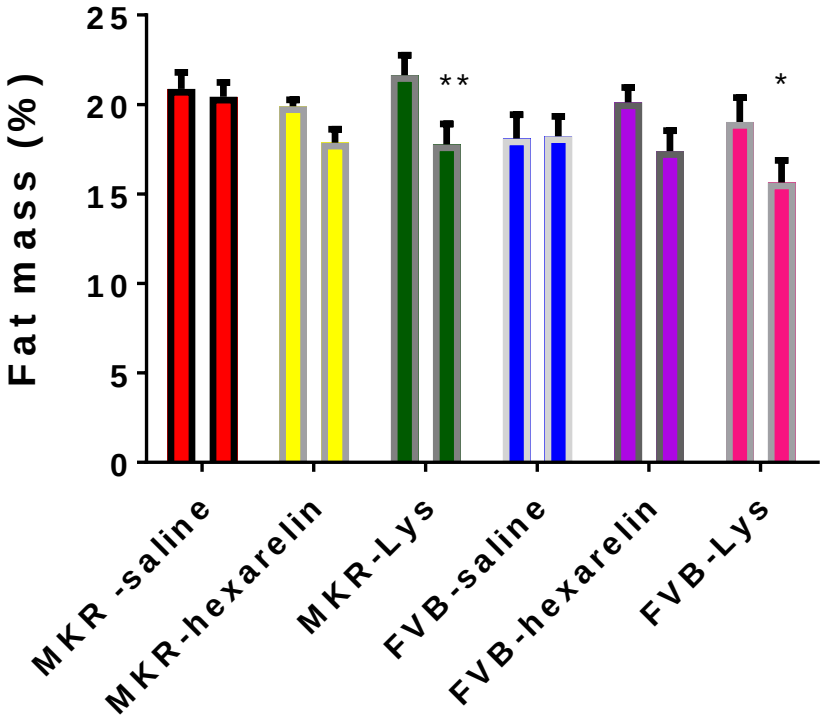


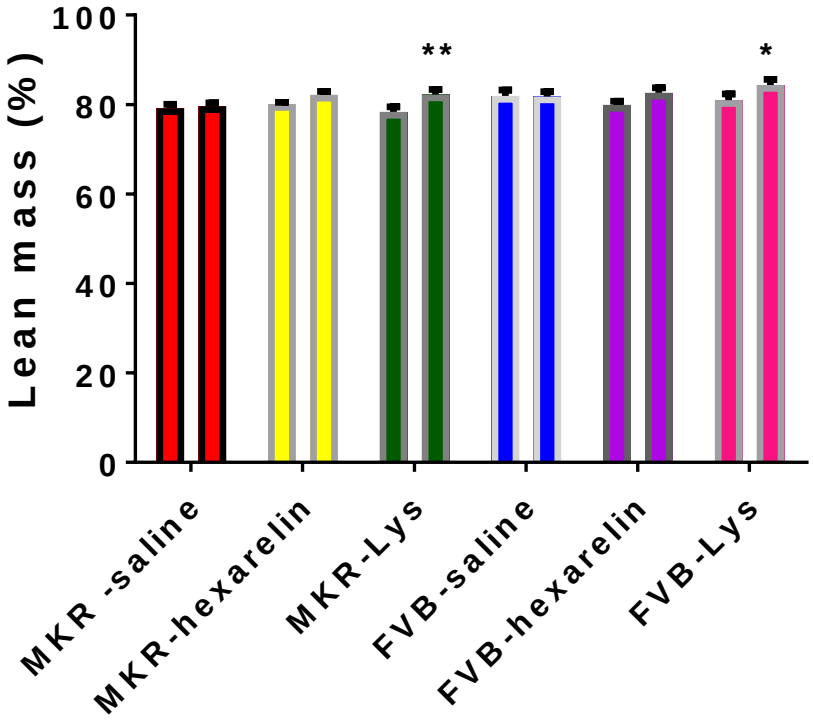
Hexarelin increased body weight in both MKR and FVB mice



The effect of hexarelin and (D-Lys)-GHRP-6 on body weight in mice (n = 5/group). Cumulative increase of body weight during a treatment period of 12 d was significant in Hexarelin treated MKR and FVB (*P <0.05,**P<0.01). One-way ANOVA was used, followed by Tukey test for multiple comparisons.

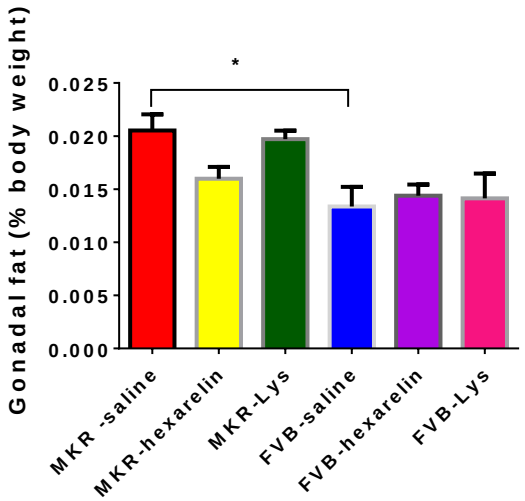
(D-Lys)-GHRP-6 decreased fat mass and increased lean mass in both MKR and FVB mice

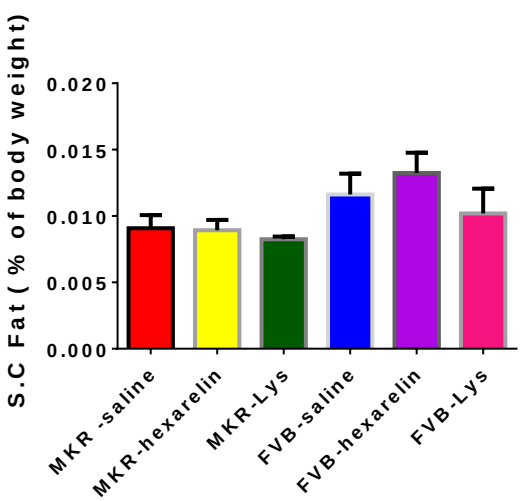


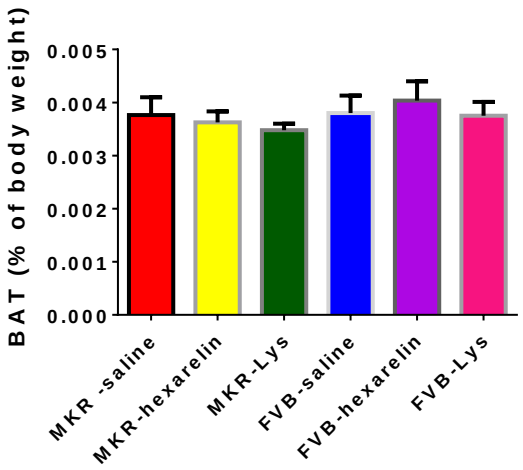


Percentage lean mass and fat mass were calculated as a proportion of the animal's total body weight using low-resolution nuclear magnetic resonance (NMR). Change of body composition after 18 d of treatment with (D-Lys)-GHRP-6 over 12 d in both MKR and FVB mice (*P <0.05,**P<0.01). One-way ANOVA was used, followed by Tukey test for multiple comparisons.

Effect of treatment on different fat tissues

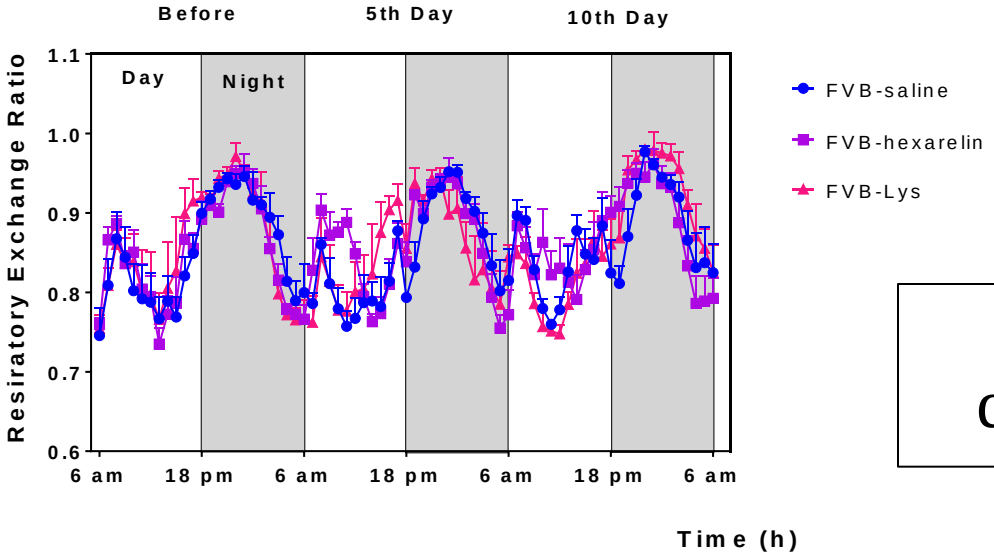




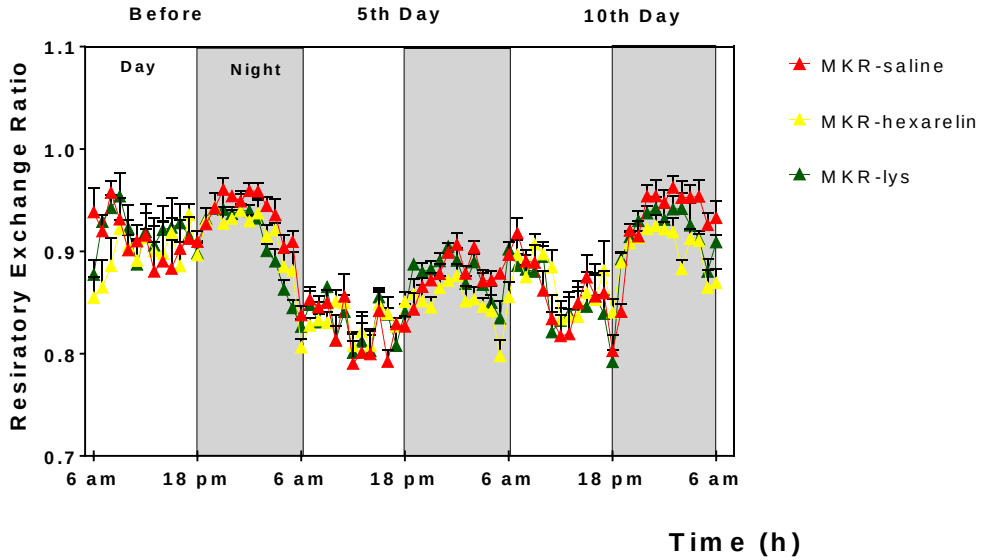


Various fat depots (Gonadal, subcutaneous and brown adipose tissues) were not affected significantly between treatment groups. However, epididymal pad weight tend to decrease by hexarelin treatment in MKR mice.

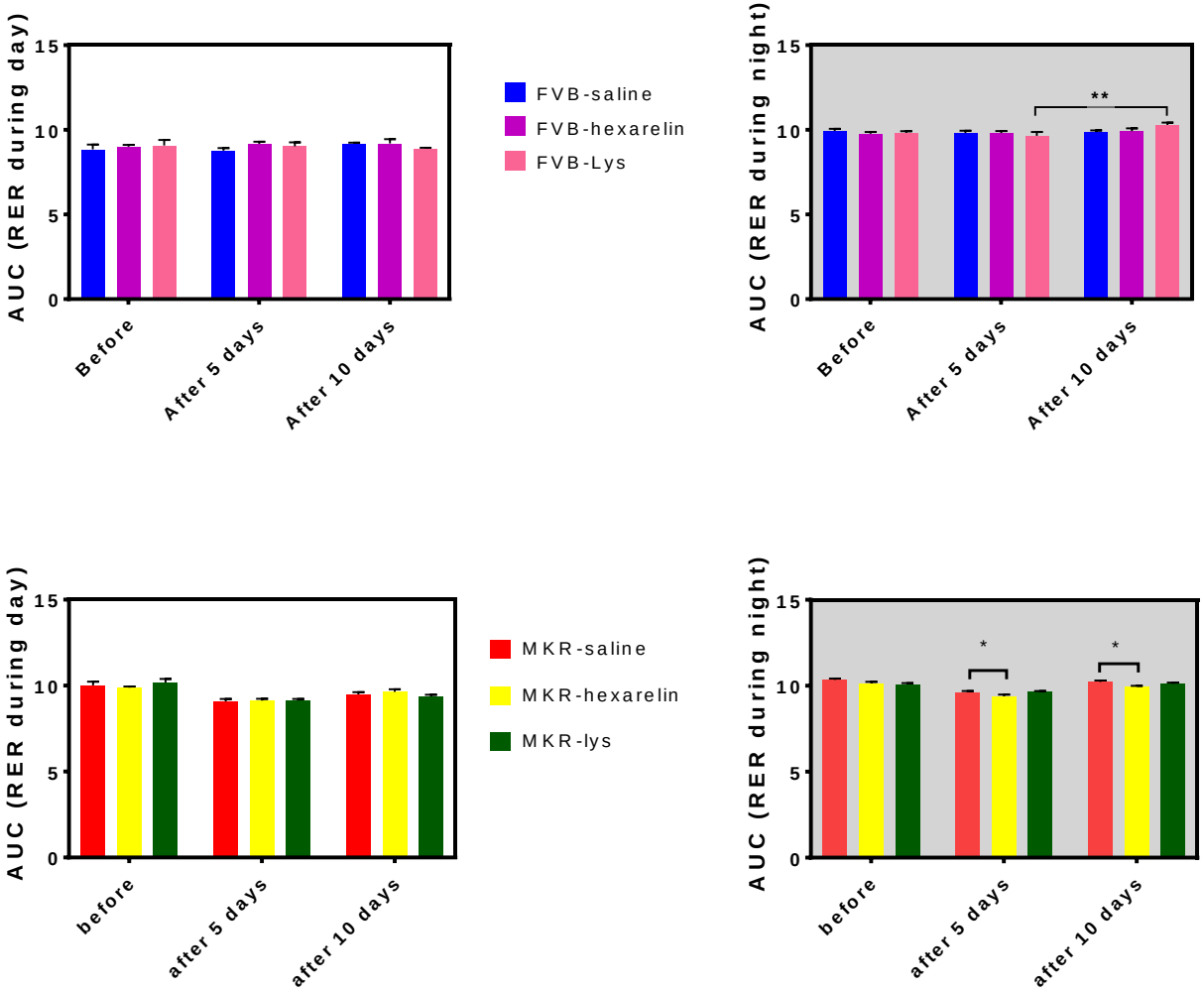
RQ were measured by indirect calorimetry



Hexarelin decreased RQ during night in MKR mice

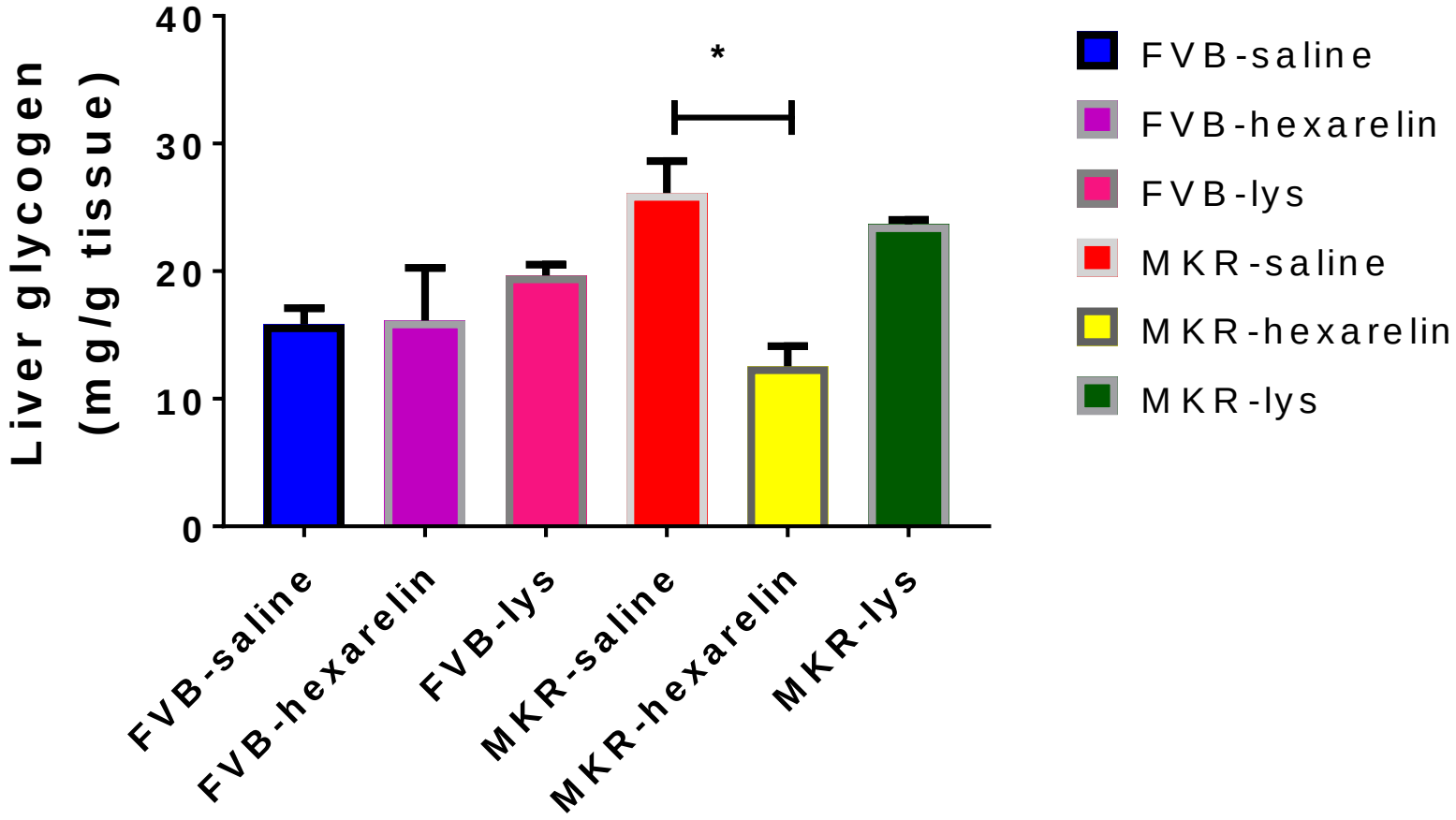


Hexarelin decreased respiratory quotient during night indicative of improved fat oxidation

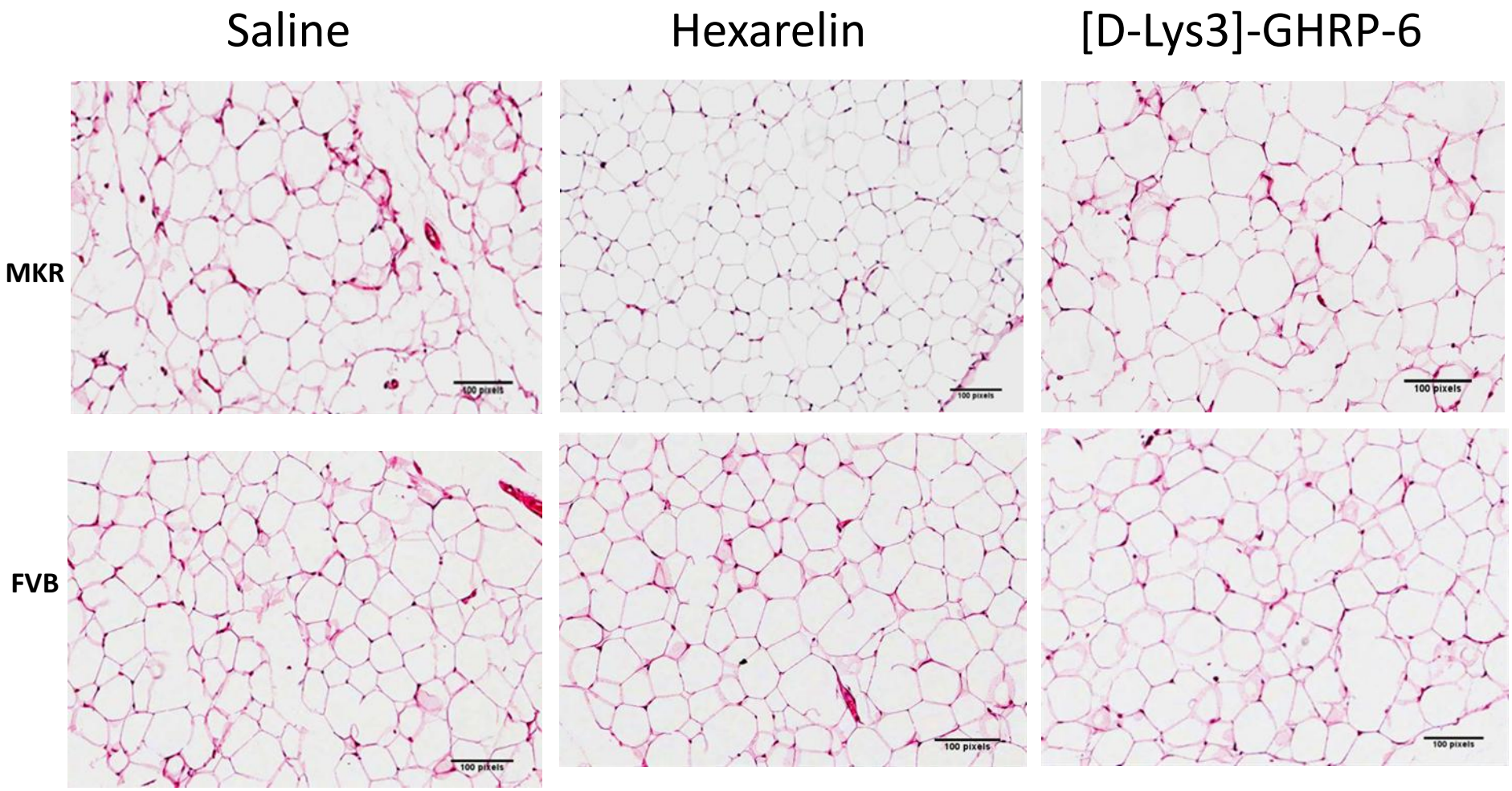


RER values plotted as Area under curve of 12-hour averages representing either dark or light periods of both MKR and FVB mice. Hexarelin treated MKR mice showed significantly decreased RQ during the dark but no significant differences during light.
In contrast, (D-Lys)-GHRP-6 treated FVB mice showed a significantly increased RQ during dark compared to other treated groups, (*P <0.05,**P<0.01).

Hexarelin decreased liver glycogen in MKR mice



Hexarelin improved adipocyte structure and differentiation in MKR mice



The size of adipocytes was smaller after hexarelin treatment in MKR mice

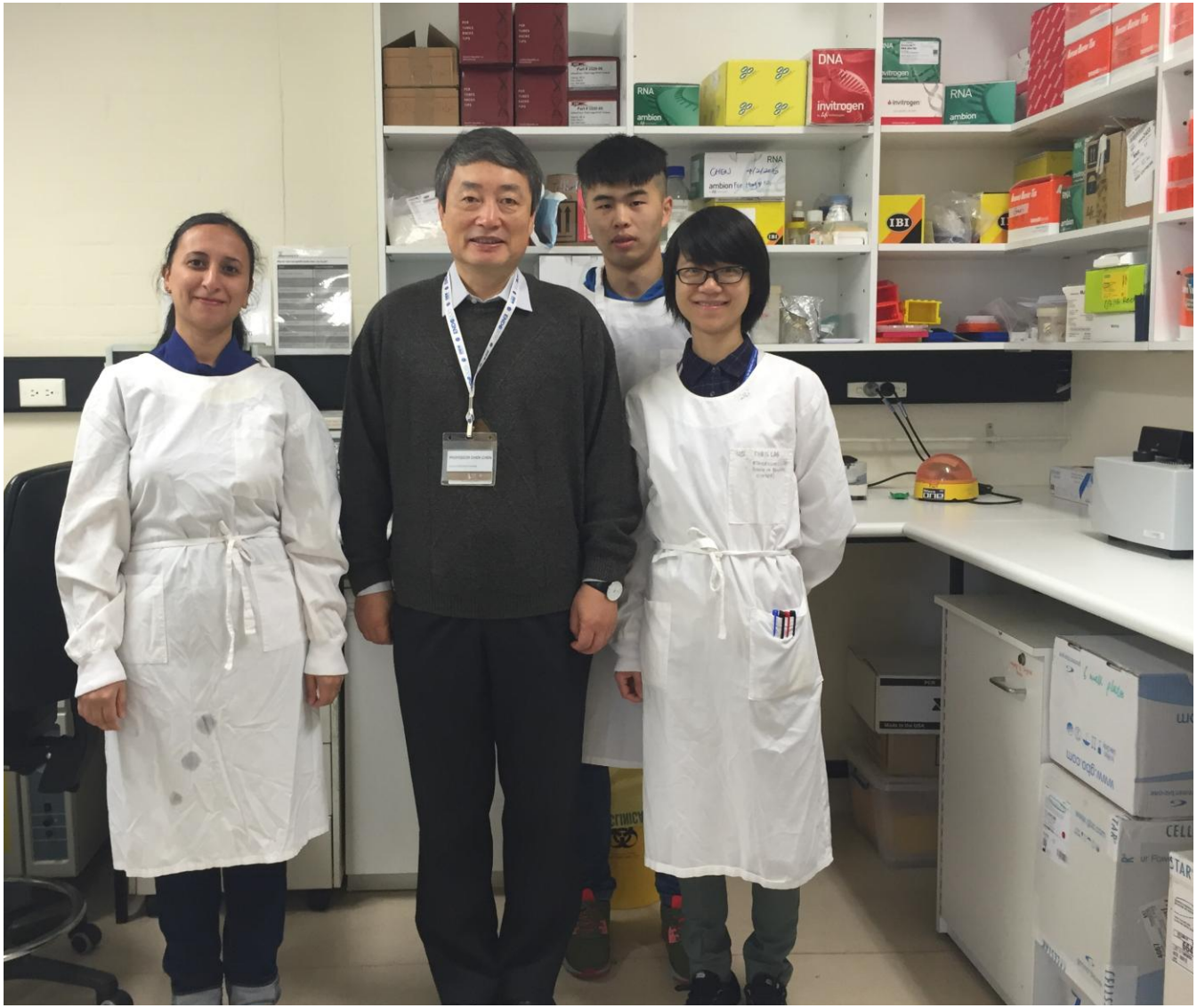
Summary

- Ectopic fat deposition plays a critical role in the pathogenesis of insulin resistance and type 2 diabetes.
- Chronic peripheral treatment with hexarelin improved glucose and insulin intolerance in MKR diabetic mice.
- Hexarelin stimulated weight gain and food intake in both wild-type and MKR mice without increasing in fat mass.
- Hexarelin-induced decrease in RQ and proliferation of adipocytes which might contribute to improving lipid utilization and insulin sensitivity in MKR diabetic mice
- Hexarelin decreased hepatic glycogen possibly through decreasing gluconeogenesis.

Conclusion

- The mechanism by which hexarelin exerts its metabolic effects represents a promising avenue which deserves further investigation to face problems related to ectopic fat deposition associated with metabolic syndrome.

Acknowledgements



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Peter Lab

Hoang Oanh Do

Miss Christine Ludick

Histological Facility

Darryl Whitehead

Arnault Gauthier

AIBN Animal House Facility

Barb Arnts

Jana Dwyer

SBMS imaging facility

Shaun Walters

Phenomaster Facility

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