Coenzyme Q_{10} prevents insulin signaling dysregulation and inflammation prior to development of insulin resistance in male offspring of a rat model of poor maternal nutrition and accelerated postnatal growth

Authors: Jane Tarry-Adkins[1], Denise Fernandez-Twinn[1], Ralitsa Madsen[1], Jian-Hua Chen[1], Asha Carpenter[1], Iain Hargreaves[2], Josie McConnell[1], and Susan Ozanne[1]

[1]University of Cambridge Metabolic Research Laboratories and MRC Metabolic Diseases Unit, Wellcome Trust-MRC Institute of Metabolic Science, Level 4, Box 289, Addenbrooke's Treatment Centre, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 OQQ, UK;

[2]Neurometabolic Unit, National Hospital, University College London, London, WC1N 3BG, UK;

Abbreviated title: CoQ prevents insulin signaling dysregulation and inflammation

Key Terms: Early life programming of adipose tissue and CoQ.

Word Count: 3169

Number of figures and tables: 10

Corresponding author and person to whom reprint requests should be addressed:

Jane Tarry-Adkins

University of Cambridge Metabolic Research Laboratories and MRC Metabolic Diseases Unit, Wellcome Trust-MRC Institute of Metabolic Science

Level 4, Box 289, Addenbrooke's Treatment Centre, Addenbrooke's Hospital

Hills Road, Cambridge, CB2 OQQ, UK

Phone: +(44) 1223 336784

Fax: +(44) 1223 330598

email: janeadkins@googlemail.com

Disclosure statement*:* The authors have nothing to disclose.

ABSTRACT:

Low birth-weight and rapid postnatal-growth increases the risk of developing insulin resistance and type-2 diabetes in later life. However underlying mechanisms and potential intervention strategies are poorly defined. Here we demonstrate that male Wistar rats exposed to a low-protein diet *in-utero* that had a low birth weight but then underwent postnatal catch-up growth (recuperated offspring) had reductions in the insulin signaling proteins p110-β [13 ± 6% of controls] ($p<0.001$) and IRS-1 [39 ± 10% of controls] ($p<0.05$) in adipose tissue. These changes were not accompanied by any change in expression of the corresponding mRNAs, suggesting post-transcriptional regulation. Recuperated animals displayed evidence of a pro-inflammatory phenotype of their adipose tissue with increased interleukin-6 (IL-6) [139 ± 8%], ($p<0.05$) and interleukin-1β (IL1-β) [154 ± 16%], ($p<0.05$) that may contribute to the insulin signaling protein dysregulation. Post-weaning dietary supplementation of recuperated animals with Coenzyme Q (CoQ_{10}) (1mg/kg of body weight/day) prevented the programmed reduction in IRS-1 and p110-β and the programmed increased in IL-6. These findings suggest that post-weaning CoQ_{10} supplementation has anti-inflammatory properties and can prevent programmed changes in insulin-signaling protein expression. We conclude that CoQ_{10} supplementation represents an attractive intervention strategy to prevent the development of insulin resistance that results from suboptimal *in-utero* nutrition.

INTRODUCTION:

Many epidemiological studies have demonstrated that a suboptimal fetal environment, resulting in low birth weight (LBW) can result in increased risk of glucose intolerance, (1, 2) insulin resistance (3) and type 2 diabetes (T2D) (4, 5) in later life. Animal models of intrauterine growth restriction (IUGR) have very similar phenotypes to LBW humans. Using a well-established model of maternal protein restriction that generates LBW offspring, we have demonstrated that the growth-restricted pups develop an insulin resistant and diabetic phenotype in later life (6). These offspring show alterations in key insulin signaling molecules in skeletal muscle and adipose tissue that are markedly similar to those observed in men with LBW (7-9). The risk for development of poor glucose tolerance (10), insulin resistance (11, 12) and T2D (13, 14) is exacerbated when LBW is combined with rapid postnatal growth. We have recently demonstrated that dysregulation of insulin signaling protein expression in adipose tissue occurs prior to the development of whole-body insulin resistance and is a very early consequence of LBW and catch-up growth (15).

Oxidative stress plays an important role in the etiology of insulin resistance (16) and in adipose tissue this is associated with increased inflammation (17). Oxidative stress accumulation is a common underlying consequence of many sub-optimal *in-utero* environments (18). Consequently, several studies have utilized antioxidant therapy during pregnancy in order to attempt to prevent the observed deleterious phenotypes resulting from a suboptimal *in-utero* environment (19 - 21). These demonstrate proof of principle that antioxidants can prevent detrimental effects of developmental programming; however, the doses employed in these studies far exceed those suitable for use in pregnant women. Furthermore, it is also important to address the potential beneficial effects of targeted postnatal antioxidant supplementation as, often evidence is not present for suboptimal *in utero* exposure until at the time of, or just after, delivery and supplementation could be detrimental to those that don't need it

Previously, we demonstrated that postnatal supplementation of coenzyme Q, also known as ubiquinone, (CoQ) at a clinically relevant dose can prevent a programmed

accelerated aging phenotype in the aorta (22) and heart (23) of rats that were born small and underwent catch-up growth. CoQ exists in various isoforms which differ in concentration between organisms. In humans, the most abundant form of CoQ is CoQ_{10} (as it contains 10 isoprenoid units attached to a benzoquinone ring); in rodents, however, CoQ_9 (containing 9 isoprenoid units) is the most abundant form. It is also known that rodents can convert dietary CoQ_{10} into CoQ_9 (23). CoQ is the most abundant and potent antioxidant in the body (24) with additional anti-inflammatory properties, including decreasing the production of pro-inflammatory cytokines (25). Clinical trials have confirmed the safety of CoQ up to doses of 1200 mg/kg/day (26, 27).

This study therefore aimed to establish whether a postnatal dietary supplementation of CoQ could alter any observed dysregulation of insulin signaling in rats exposed to a suboptimal environment *in-utero* and postnatal catch-up growth and then to investigate the potential underlying mechanisms, including oxidative stress status and inflammatory phenotypes.

MATERIALS AND METHODS:

Animal Experimentation

All procedures involving animals were conducted under the British Animals (Scientific Procedures) Act (1986). Pregnant Wistar rats were maintained on a 20% protein diet (control) or an isocaloric low-protein (LP) (8%) diet fed *ad libitum*, as previously described (28). Both diets were purchased from Arie Blok, The Netherlands. The day of birth was recorded as day 1 of postnatal life. Pups born to LP diet-fed dams were cross-fostered to control-fed mothers on postnatal day 3 in order to create a recuperated litter. Each recuperated litter was culled to 4 male pups at random to maximize their plane of nutrition. The control group constituted offspring of mothers fed the 20% protein diet and suckled by 20% protein fed dams. Each control litter was culled to 8 pups as a standard. To prevent any stress to the animals during cross-fostering, pups were transferred with their own bedding.. At 21 days, two males per litter were weaned onto standard laboratory chow (Special Diet Services) and the other two were weaned onto the same diet supplemented with CoQ to give a dose of 1 mg/kg body weight/day. Animals were maintained on these diets until 3 months of age. Body weights were recorded at postnatal days 3, 7, 14, 21 and 3 months of age. All animals were killed by CO_2 asphyxiation. At post mortem, epididymal fat pads, liver and vastus lateralis skeletal muscle were removed, weighed, and snap frozen in liquid nitrogen and then stored at -80 ºC until analysis. For all measurements, one pup per litter was used, thus N indicated throughout represents number of litters. Only male animals were used in this study.

CoQ diet preparation

A dose of 1 mg CoQ /kg of body weight per day was used in this study. This was achieved by appropriate CoQ supplementation of laboratory chow as described previously (22, 23). Briefly, CoQ was impregnated into the diet pellets by dissolving CoQ_{10} in acetone and mixing this with the diet pellets. The mix was then left in a fume hood overnight to allow evaporation of acetone. The diet was prepared twice a week throughout the study.

CoQ_{10}, lipid profile, glucose and insulin analysis

Serum was obtained from blood collected from the tail vein after overnight fasting. The blood clotted for 30 minutes before centrifugation for 3 minutes at 845 g. Fasted blood glucose measurements were obtained using a blood glucose analyser (Hemocue). The lipid profile and fasted serum insulin analysis was performed using an auto analyzer (Clinical Chemistry Laboratory, Medical Research Council Centre for Obesity and Related Metabolic Diseases, Cambridge, UK). Blood from the tail vein was collected into heparin tubes and centrifuged to isolate plasma. Plasma CoQ_{10} levels were determined by reverse-phased HPLC with UV detection at 275 nm as previously described (22).

Protein analysis

Protein was extracted and assayed as described previously (29) and 20 μg protein was loaded onto 10-15% polyacrylamide gels, dependent upon the molecular weight of the target protein. The samples were then electrophoresed and transferred to polyvinylidene fluoride membranes (22) and antibodies to the following proteins were detected: IL-6, IL1β (Abcam), IRS-1, p110-β, p85α (Merck Millipore), Akt-1, (Cell Signaling Technology), and Akt-2, pAkt $^{(ser473)}$ (New England Biolabs). (Please see the Antibody table for further details). Anti-rabbit and anti-mouse IgG horseradish peroxidase-linked secondary antibodies were from Jackson Immunoresearch Laboratories. Equal protein loading was confirmed by staining electrophoresed gels with Coomassie Blue (Bio-Rad, Hemel Hempstead, Hertfordshire, UK) to visualize total protein. To ensure the chemiluminescent signal changed in a linear manner, the ratio between loading controls (50% and 100% pooled sample) was confirmed for each detected protein. Protein bands were detected using West Pico Chemiluminescence reagent (Pierce, Thermo Scientific) and analyzed using Alphaease Imaging Software (Alpha Innotech).

Gene expression

RNA was extracted using a miRNeasy mini kit (Qiagen) following the manufacturers instructions (22), a DNAse digestion step was performed in order to ensure no gDNA contamination. RNA (1 ug) was used to synthesize cDNA using oligo-dT primers and M-MLV reverse transcriptase (Promega, Southampton, Hants, UK). Gene expression was determined using custom designed primers (Sigma) and SYBR Green reagents (Applied Biosystems). (Primer sequences are described in Table 1).

Quantification of gene expression (expressed as average copy number) was performed using the Step One Plus q-PCR machine (Applied Biosystems). Melting curve analysis was performed to confirm absence of primer-dimers. Equal efficiency of the reverse transcription of RNA from all groups was confirmed through quantification of expression of the housekeeping gene *β-actin*. Expression did not differ between groups (effect of maternal diet p=0.72; (control; 153 ± 32, recuperated; 134 ± 35 average copy number).

microRNA (miRNA) analysis

Putative miRNAs targeting the 3' untranslated regions (3'UTRs) of *p110β* and *IRS-1* were identified using the miRanda/mirSVR 1.2 (Release 2010) and miRmap 3 (Release 1.1) prediction algorithms, respectively. Candidates were ranked according to the strength of the predicted interaction, high conservation across species and target site position along the mRNA (higher scores for proximal and distal locations). The final selection of the most highly ranked candidates to assess was based on common target sites within both the *p110β* and *IRS-1* 3'UTRs as well as information regarding their expression in white adipose tissue. RNA was extracted using a Direct-zol™ RNA MiniPrep kit (Zymo Research), in order to retain RNA species with a minimal length of 17 nucleotides. RNA purity and concentration was determined by spectrophotometric analysis on a NanoDrop ND-1000. RNA integrity was confirmed by denaturing agarose gel electrophoresis.

Statistical Analysis

Data were analyzed using a 2-way ANOVA with maternal diet and CoQ supplementation as the independent variables. Data are represented as mean ± S.E.M. A value of $p<0.05$ was considered statistically significant. All statistical analyses were performed using Statistica 7 software (Statsoft Inc) except for miRNA analysis where GraphPad Prism 6.0 (GraphPad) was utilised. In all cases, N refers to the number of litters.

RESULTS:

Physical parameters, plasma CoQ, lipid profile and insulin data

Recuperated offspring were born smaller [(6.3 ± 0.2g vs. 7.4 ± 0.2g); ($p<0.001$)] than control animals and remained smaller at postnatal day 7 [(13.4g ± 0.4 vs. 16.4g ± 0.4); ($p<0.001$)]. By postnatal days 14 (33.7 ± 0.5g vs. 33.3 ± 0.8g) and 21 (52.2 ± 3.7 vs. 50.8 ± 1.2g), the recuperated offspring had undergone accelerated catch-up growth and were therefore of similar weight to the control offspring. At 3 months of age, there was no significant effect of CoQ supplementation and maternal diet on offspring body or liver weights (Table 2). However, maternal diet increased ($p<0.05$) epididymal fat pad weight (Table 2). There was no significant effect of CoQ supplementation on epididymal fat pad weight. Furthermore, no significant effect of maternal diet upon offspring plasma CoQ levels was observed. However, plasma CoQ levels were significantly ($p<0.01$) increased by CoQ supplementation (Figure 1). Serum levels of fasting insulin, triglycerides, free fatty acids, total cholesterol and fasting plasma glucose were unaltered by maternal diet or CoQ supplementation and there was no interaction between these two variables on any of the serum levels (Table 3).

Insulin signaling: protein, mRNA and miRNA analysis

a) Effect of maternal diet: Protein expression of IRS-1 ($p<0.05$) and p110-β ($p<0.001$) were reduced in the epididymal fat pads of recuperated offspring compared to controls (Figure 2A). In contrast, PKC-ζ protein levels were significantly ($p<0.05$) increased in the recuperated group (Figure 2A). Gene (mRNA) expression of these molecules were unaffected by maternal diet (Figure 3A). In liver and vastus lateralis skeletal muscle, IRS-1 and p110-β protein expression remained unaltered by maternal diet. However, liver PKC-ζ protein levels displayed an increase ($p<0.001$) (Figures 2B and 2C). The mRNA levels of these three genes were not different in either the liver or muscle of recuperated offspring when compared to controls (Figures 3B and 3C). Protein expression of other insulin signaling

molecules including IRβ, p85α, Akt-1, Akt-2, pAkt$^{(Ser473)}$, and GLUT-4 were unaffected by maternal diet in the epididymal fat, liver and vastus lateralis muscle (Table 4).

The programmed reduction in adipose tissue IRS-1 and p110-β in the absence of any differences in the corresponding mRNAs suggested that this effect was mediated by a post-transcriptional mechanism. However, we saw no effects of maternal diet on the five miRNAs predicted to regulate *p110-β* (miR-25, miR-301a, miR-19a, miR-130a and miR-130b) or the two predicted to regulate IRS-1 (miR-126 and miR-143), (Figures 4A and B).

b) Effect of CoQ supplementation: CoQ supplementation significantly increased both IRS-1 and p110-β protein levels in recuperated offspring epididymal fat. In control offspring, CoQ supplementation had no effect on p110-β levels; however supplementation increased IRS-1 protein expression (Figure 2A). CoQ supplementation increased ($p<0.001$) p110-β protein expression in both liver and VL skeletal muscle (Figures 2B and C). CoQ supplementation significantly ($p<0.001$) decreased PKC-ζ protein levels in recuperated adipose tissue compared to the effect on control offspring (Figure 2A); however, PKC-ζ expression remained similar in the liver and vastus lateralis muscle (Figures 2B and C).

Cytokine analysis

Adipose tissue protein expression of IL-6 ($p<0.05$) and IL-1β ($p<0.01$) were increased in recuperated offspring (Figure 5A). CoQ supplementation significantly ($p<0.05$) reduced IL-6 levels back to that of the controls, however, IL-1β levels were unchanged by CoQ supplementation (Figure 5 A). mRNA levels of *Tgfb1* and *Lep* were unaltered by maternal diet; however, CoQ supplementation reduced *Tgf-βl* ($p<0.001$) and *Lep* ($p<0.05$) levels (Figure 5B). *Tnf-α* and *Mcp-1* gene expression were similar between all groups (Figure 5B). There was no significant effect of maternal diet or CoQ_{10} supplementation on adiponectin (*adipoq)* mRNA in epididymal fat (control: 100 ± 26.6%; recuperated: 107 ± 18%; control CoQ: 91 ± 32%; recuperated CoQ: 73 ± 9%).

230 Reactive oxygen species (ROS) and antioxidant defense capacity

231 4-HNE (4-hydroxynoneal; a marker of lipid peroxidation) and 3-NT (3-nitrotyrosine;
232 a measure of protein tyrosine nitration) were both undetectable in epididymal adipose tissue
233 (*data not shown*). No significant effect of maternal diet or CoQ supplementation was
234 observed upon xanthine oxidase (XO) protein expression (Figure 6A). mRNA levels of
235 components of NADPH oxidase-2 (NOX-2) ($Gp91^{phox}$ and $P22^{phox}$) were unchanged by
236 maternal diet; however, CoQ supplementation reduced $Gp91^{phox}$ ($p<0.001$) and $P22^{phox}$
237 ($p<0.01$) levels, (Figure 6B). No significant effect of maternal diet or CoQ supplementation
238 was observed upon manganese superoxide dismutase (MnSOD) or glutathione reductase (GR)
239 protein expression (Figure 6C). Catalase protein expression was significantly ($p<0.01$)
240 increased in recuperated offspring compared to controls (Figure 6C), and this effect remained
241 unaffected by CoQ supplementation.
242

DISCUSSION:

Previous studies by us and others have demonstrated that adipose tissue is very vulnerable to the effects of developmental programming and is the site wher some of the earliest and most striking programming effects are observed (15, 30, 31). Consistent with these observations, in the current study, we have shown a reduction in IRS-1 and p110-β insulin signaling protein expression in response to suboptimal nutrition *in utero* and catch-up growth. In contrast, maternal diet increased protein expression of PKC-ζ in adipose tissue and liver, which can negatively regulate insulin signaling via serine phosphorylation of IRS-1 (32). All of these differences are therefore consistent with an insulin resistant phenotype. These alterations are present in adipose tissue prior to any such defects in liver or skeletal muscle. The differences in adipose tissue insulin signaling proteins are present at a time when animals display no difference in fasting glycaemia or insulinaemia. These defects are therefore not a consequence of metabolic dysfunction and more likely a contributor to the increased risk of insulin resistance in the recuperated offspring (33).

The changes in p110β and IRS-1 protein expression were not accompanied by altered mRNA expression, which suggests the involvement of posttranscriptional regulatory mechanisms. However, none of the seven candidate miRNAs predicted to regulate translation of IRS-1 and/or p110β were differentially expressed in offspring epididymal adipose tissue in response to maternal diet. These could mean that a simple concentration change of complementary miRNAs is not the cause of altered protein expression of IRS-1 and p110-β, or that other undefined miRs or other posttranscriptional mechanisms are involved.

A clinically relevant dietary supplementation of CoQ normalized the defects in adipose tissue insulin signaling protein expression in recuperated offspring. In addition, CoQ supplementation increased p110β protein expression in liver and skeletal muscle. This is consistent with previous studies showing that administration of a much higher dose of CoQ (20 mg/kg) affects insulin sensitivity and has anti-diabetic properties via increasing the activity of phosphatidylinositol kinase (PI3K) in the liver and skeletal muscle of rats fed a high-fat, high-fructose diet (34). It is unclear how CoQ supplementation has its effects on

insulin signaling protein expression. However, uptake of CoQ into most tissues is thought to be low (35) and a systemic effect is likely to be involved.

Inflammation is an important factor in the development of insulin resistance via inhibition of insulin signaling through activation of IKK-β and JNK pathways (36). IL-6, IL-1β, tumor necrosis factor-α (TNF-α), monochemoattractant protein-1 (MCP-1), transforming factor-β1 (TGF-β1) and leptin are major cytokines and chemokines that can be secreted by dysfunctional adipocytes or infiltrated adipose tissue macrophages. These factors play a pivotal role in adipose tissue-induced low-grade systemic inflammation and/or obesity. In the current study, we demonstrated increased protein expression of IL-6 and IL-1β in the epididymal fat pads of recuperated offspring. IL-6 is known to inhibit protein and mRNA expression of IRS-1 (37), suggesting that the increased IL-6 levels could, at least in part, explain the observed reduction in IRS-1 protein levels in recuperated animals. Furthermore, IL-1β is a key factor in mediating macrophage-induced insulin resistance in human adipocytes (38). However Mcp-1 expression (a key chemokine that regulates migration and infiltration of monocytes and macrophages) was unaltered between groups, suggesting that any macrophage infiltration in recuperated adipose tissue could be via an Mcp-1 independent mechanism. Interestingly, this pro-inflammatory phenotype occurred in the absence of obesity, suggesting that maternal suboptimal nutrition is the driving factor for this effect in a manner that is independent of offspring adiposity. CoQ supplementation significantly reduced IL-6 protein and *Tgf-β1* and *Lep* mRNA levels, which is in agreement with findings in cardiac tissue (25), and CoQ is known to have anti-inflammatory properties in mouse liver (39) and human plasma (40) however, to our knowledge this is the first time that CoQ has been demonstrated to have anti-inflammatory properties in adipose tissue. This highlights the function of CoQ as an important anti-inflammatory molecule and may partially explain some of our reported 'insulin sensitizing' effects of CoQ.

Over-production of reactive oxygen species (ROS) is well-known to contribute to the development of insulin resistance. In the current study, however, no evidence of an oxidative stress phenotype was observed in the adipose tissue of recuperated animals, given that

299 *Gp91*^{phox}, *P22*^{phox} and xanthine oxidase (all important sources of ROS) were unaltered by
300 maternal diet and indicators of oxidative stress such as 4-HNE and 3-NT were undetectable.
301 This suggests that the manifestation of insulin resistance in recuperated offspring may be
302 driven by inflammation and not oxidative stress at this age. Previously, we have described an
303 oxidative stress phenotype in other tissues from recuperated offspring adipose tissue at 3
304 months of age, including pancreatic islets (41) and the heart (23), and its absence in the
305 adipose tissue highlights the tissue-specificity of developmental programming on the
306 manifestation of oxidative stress. mRNA levels of *Gp91*^{phox} and *P22*^{phox} were significantly
307 decreased by CoQ supplementation, demonstrating that CoQ has a role as a potent antioxidant
308 in adipose tissue.
309 In conclusion, a suboptimal maternal environment and rapid postnatal catch-up growth
310 initiates dysregulation of insulin signaling protein expression in epididymal adipose tissue
311 that may contribute to later development of whole body insulin resistance. This was
312 associated with increased expression of inflammatory markers, independent of increased ROS
313 generation and independent of obesity. CoQ supplementation ameliorated both insulin
314 signaling dysfunction and inflammation in the adipose tissue of 'recuperated' offspring,
315 suggesting that CoQ's anti-inflammatory actions may play some role in modulating insulin
316 resistance. Although the mechanism through which CoQ mediates these changes in adipose
317 tissue is currently unknown, it is plausible that CoQ could act via antioxidant mechanisms to
318 impact on the activity of genes involved in regulation of transcription or translation.

FIGURE LEGENDS:

Figure 1 - The effect of *in utero* protein restriction, accelerated postnatal growth and CoQ supplementation upon plasma CoQ levels in 3-month-old male rats. Results are expressed as mean ± S.E.M. * $p<0.01$ (C and R versus CQ and RQ). C = control; CQ = control CoQ; R = recuperated; RQ = recuperated CoQ. N = 10 per group.

Figure 2 - The effect of *in utero* protein restriction, accelerated postnatal growth and CoQ supplementation upon protein expression of insulin signaling molecules in A) epididymal adipose tissue, B) liver and C) VL skeletal muscle in 3-month-old male rats. Results are expressed as mean ± S.E.M. * $p<0.01$ and *** $p<0.001$ (C versus R) and * $p<0.01$ and *** $p<0.001$ (R vs RQ). C = control; CQ = control CoQ; R = recuperated and RQ = recuperated CoQ. N = 5 - 6 per group.

Figure 3 - The effect of *in utero* protein restriction and accelerated postnatal growth upon gene expression of insulin signaling molecules in A) epididymal adipose tissue, B) liver and C) VL skeletal muscle in 3-month-old male rats. Results are expressed as mean ± S.E.M. N = 8 per group.

Figure 4 - The effect of *in utero* protein restriction and accelerated postnatal growth upon miRNA targets to A) *p110β* and B) *IRS-1*. Results are expressed as mean ± S.E.M. N = 10 per group.

Figure 5 - The effect of *in utero* protein restriction, accelerated postnatal growth and CoQ supplementation upon cytokine protein and gene expression in epididymal adipose tissue in 3-month-male rats. Results are expressed as mean ± S.E.M. * $p<0.05$ (C versus R) and * $p<0.05$ (R vs RQ). C = control; CQ = control CoQ; R = recuperated and RQ = recuperated CoQ. N = 5 - 6 per group.

Figure 6 - The effect of *in utero* protein restriction, accelerated postnatal growth and CoQ supplementation upon A) and B) sources of ROS and C) antioxidant defense capacity in 3-month-old male rat epididymal adipose tissue. Results are expressed as mean ± S.E.M. *

$p<0.05$ (C and R versus CQ and RQ). C = control; CQ = control CoQ; R = recuperated and RQ = recuperated CoQ. N = 6 per group.

ACKNOWLEDGMENTS:

The authors would like to thank Professor Ken Siddle for helpful discussion. This work was supported by The British Heart Foundation [PG/09/037/27387, FS/09/029/27902]; Medical Research Council [MC_UU_12012/4] and Diabetes UK [12/0004508]. SEO is a member of the MRC Metabolic Diseases Unit. IPH is supported by the Department of Health's NIHR Biomedical Research Centres funding scheme at UCLH/UCL.

355 **REFERENCES:**

356 1. Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, Winter PD. Fetal and infant
357 growth and impaired glucose tolerance at age 64. BMJ 1991; 303:1019–1022.

358 2. Ravelli AC.J, van der Meulen JHP, Michels RPJ, Osmond C, Barker DJP, Hales CN,
359 Bleker OP. Glucose tolerance in adults after prenatal exposure to famine. Lancet 1998; 351:
360 173–177.

361 3. Ong KK, Petry CJ, Emmett P.M, Sandhu MS, Kiess W, Hales CN, Ness AR, Dunger, DB.
362 ALSPAC study team. Insulin sensitivity and secretion in normal children related to size at
363 birth, postnatal growth and plasma insulin-like growth factor-I levels. Diabetologia 2004; 47:
364 1064–1070.

365 4. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM. Type 2 (non-insulin-
366 dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to
367 reduced fetal growth. Diabetologia 1993; 36: 62–67.

368 5. Lithell HO, McKeigue PM, Berglund L, Mohsen R, Lithell UB, Leon DA. Relation of size
369 at birth to non-insulin dependent diabetes and insulin concentrations in men aged 50–60
370 years. BMJ 1996; 312: 406–410.

371 6. Petry CJ, Dorling MW, Pawlak DB, Ozanne SE, Hales CN. Diabetes in old male offspring
372 of rat dams fed a reduced protein diet. Int J Exp Diabetes Res 2001; 2: 139–143.

373 7. Ozanne SE, Jensen CB, Tingey KT, Storgaard H, Madsbad S, Vaag, AA. Low birth weight
374 is associated with specific changes in muscle insulin signalling protein expression.
375 Diabetologia 2005; 48: 547–552.

376 8. Ozanne SE, Olsen GE, Hansen LL, Tingey KT, Nave BT, Wang CL, Hartil K, Petry CJ,
377 Buckley A, Mosthaf-Seedorff L. Early growth restriction leads to down regulation of protein
378 kinase C zeta and insulin resistance in skeletal muscle. J. Endocrinol 2003; 177: 235–241.

9. Ozanne SE, Jensen CB, Tingey KJ, Martin-Gronert MS, Grunnet L, Brons C, Storgaard H, Vaag AA.Decreased protein levels of key insulin signalling molecules in adipose tissue from young men with a low birth weight: potential link to increased risk of diabetes? Diabetologia 2006; 49: 2993-2999.

10. Crowther NJ, Cameron N, Trusler J, Gray IP. Association between poor glucose tolerance and rapid postnatal weight gain in seven year old children. Diabetologia 1998; 41: 1163–1167.

11. Mericq, V., Ong, K. K., Bazaes, R., Pena, V., Avala, A., Salasar, T., Soto, N., Iniguez, G., and Dunger, D. B. (2005) Longitudinal changes in insulin sensitivity and secretion from birth to age three years in small- and appropriate-for-gestational-age children. Diabetologia; 48: 2609–2614.

12. Dellschaft#NS, Alexandre-Gouabau#MC, Gardner DS, $ı-ŦŁı˚″#JP, #Keisler DH, Budge H, Symonds ME, Sebert SP. Effect of pre- and postnatal growth and post-weaning activity on glucose metabolism in the offspring. J. Endocrinol 2015; 224: 171-182.

13. Forsen T, Eriksson J, Tuomilehto J, Reunanen A, Osmond C, Barker D. The fetal and childhood growth of persons who develop type 2 diabetes. Ann Intern Med; 2000: 133: 176–182.

14. Yajnik CS. Early life origins of insulin resistance and type 2 diabetes in India and other Asian countries. J Nutr 2004; 134; 205–210.

15. Berends LM, Fernandez-Twinn DS, Martin-Gronert MS., Ozanne, SE. Catch-up growth following intra-uterine growth-restriction programmes an insulin-resistant phenotype in adipose tissue. Int J Obes 2013; 8: 1051-1057.

16. Henrikse, EJ, Diamond-Stanic MK, Marchionne EM. Oxidative stress and the etiology of insulin resistance and type 2 diabetes. Free Radic. Biol. Med 2011; 51: 991-999.

403 17. Francisco L, Torres-Leal, Miriam H. Fonseca-Alaniz, Ariclécio Cunha de Oliveira and
404 Maria Isabel C. Alonso-Vale (2012). Adipose Tissue Inflammation and Insulin Resistance,
405 Insulin Resistance, Dr. Sarika Arora (Ed.), ISBN: 978-953-51-0890-0, InTech, DOI:
406 10.5772/53974.
407 18. Tarry-Adkins JL, Ozanne, SE. The impact of early nutrition on the ageing trajectory. Proc
408 Nutr Soc 2014; 2: 289-301.
409 19. Sen S, Simmons RA Maternal antioxidant supplementation prevents adiposity in Western
410 diet fed rats. Diabetes 2010; 59: 3058-3065.
411 20. Giussani DA, Camm EJ, Nui Y, Richter HG, Blanco CE, Gottschalk R, Blake EZ, Horder
412 KA, Thakor AS, Hansell JA, Kane AD, Wooding FB, Cross CM Herrera EA. Developmental
413 programming of cardiovascular dysfunction by prenatal hypoxia and oxidative stress. PLoS
414 One 2012; 2: e31017.
415 21. Cambonie G, Comte B, Yzdorczyk C, Ntimbane T, Germaine N, Le NL, Pladys P,
416 Gauthier C, Lahaie I, Abram D, Lavoie JC, Nuyt AM. Antenatal oxidant prevents adult
417 hypertension, vascular dysfunction, and microvascular rarefaction associated with in utero
418 exposure to a low-protein diet. Am J Regul Interg Comp Physiol 2007; 292: R1236-R1245.
419 22. Tarry-Adkins JL, Fernandez-Twinn DS, Chen JH, Hargreaves IP, Martin-Gronert MS,
420 McConnell JC, Ozanne SE. Nutritional programming of coenzyme Q: potential for prevention
421 and intervention? FASEB J 2014; 28: 5398-5405.
422 23. Tarry-Adkins JL, Blackmore HL, Martin-Gronert MS, Fernandex-Twinn DS, McConnell
423 JM, Hargreaves, IP, Ozanne SE. Coenzyme Q prevents accelerated cardiac aging in a rat
424 model of poor maternal nutrition and accelerated postnatal growth. Mol Metab 2013; 2: 480-
425 490.
426 24. Littarru, GP, Tiano L. Bioenergetic and antioxidant properties of coenzyme Q10: recent
427 developments. Mol Biotechnol 2007; 37, 31-37.

428 25. Lee BJ, Tseng YF, Yen CH, Lin PT. Effects of coenzyme Q10 supplementation (300mg/
429 day) on antioxidation and anti-inflammation in coronary artery disease patients during statins
430 therapy: a randomized, placebo-controlled trial. Nutr J 2013; 12: doi: 10.1186/1475-2891-12-
431 142.

432 26. Hidaka T, Fuji K, Funahashi I, Fukutomi N, Hoseo K Safety assessment of coenzyme Q_{10}
433 (CoQ_{10}). Biofactors 2008; 32: 199-208.

434 27. Ikematsu H, Nakamura K, Harashima S, Fujii K, Fukutomi N. Safety assessment of
435 coenzyme Q10 (Kaneka Q10) in healthy subjects: A double-blind, randomized placebo
436 controlled trial. Regul Toxicol Pharmacol 2006; 44: 212-218.

437 28. Snoeck A, Remacle C, Reusens B, Hoett JJ. Effect of low protein diet during pregnancy
438 on the fetal rat endocrine pancreas. Biol Neonate 1990; 57: 107-118.

439 29. Tarry-Adkins JL. Joles JA, Chen JH, Martin-Gronert MS, van der Giezen DM,
440 Goldschmeding R, Hales CN and Ozanne SE. Protein restriction in lactation confers
441 neproprotective effects in the male rat and is associated with increased antioxidant protein
442 expression. Am J Physiol Regul Integr Comp Physiol 2007; 293:R1259-1266.

443 30. Mackay H, Khazall R, Patterson ZR, Wellman M, Abizaid A. Rats perinatally exposed to
444 food restriction and high-fat diet show differences in adipose tissue gene expression under
445 chronic caloric restriction. Adipocyte 2013; 4: 237-245.

446 31. Schenk S, Saberi M, Olefsky JM. Insulin sensitivity: modulation by nutrients and
447 inflammation. JCI 2008; 118: 2992-3002.

448 32. Guilherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to
449 insulin resistance and type 2 diabetes. Nat. Rev. Mol. Cell Biol.2008; 5: 367-377.

450 33. Ravichandran LV, Esposito DL, Chen J, Quon MJ. Protein kinase C-ζ phosphorylates
451 insulin receptor substrate-1 and impairs its ability to activate phosphatidylinositol 3-kinase in
452 response to insulin J Biol Chem 2001; 276: 3543-3549.

453 34. Amin MM, Asaad GF, Abdel-Salem RM, El-Abhar HS., Arbid MS. Novel CoQ10
454 antidiabetic mechanisms underlie its positive effect: Modulation of insulin and adiponectine
455 receptors, tyrosine kinase, PI3K, glucose transporters, sRAGE, and visfatin in insulin
456 resistant/diabetic rats. PLoS ONE 2014; 9: e89169.

457 35. Lonnrot K, Holm P, Lagerstedt A, Huhtala H, Alho H. The effects of lifelong ubiquinone
458 supplementation on the CoQ9 and CoQ10 tissue concentrations and lifespan of male rats and
459 mice. Biochem Mol Biol Int 1998; 44: 727-737.

460 36. McNelis JC, Olefsky, JM Macrophages, immunity and metabolic disease. Immunity 2014;
461 41: 36-48.

462 37. Rotter V, Nagaev I, Smith U. Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1
463 adipocytes and is, like IL-8 and tumor necrosis factor-α, overexpressed in human fat cells
464 from insulin-resistant subjects. J Biol Chem (2003; 46: 45777-45784.

465 38. Gao D, Madi M, Ding C, Fok M, Steele T, Ford C, Hunter L, Bing C. Interleukin-1-β
466 mediates macrophage-induced impairment of insulin signaling in human primary adipocytes.
467 Am J Physiol Endocrinol Metab 2014; 307: E289-E304.

468 39. Sohet FM, Neyrinck AM, Pachikian BD, de Backer FC, Bindels LB, Niklowitz P, Menke
469 T, Cani PD, Delzenne NM. Coenzyme Q10 supplementation lowers hepatic oxidative stress
470 and inflammation associated with diet-induced obesity in mice. Biochem Pharmacol
471 2009;78:1391-1400.

472 40. Sanoobar M, Eghtesadi S, Azimi A, Khalili M, Khodadi B, Jazayeri S, Gohari
473 MR, Aryaeian N. Coenzyme Q10 supplementation ameliorates inflammatory markers
474 in patients with multiple sclerosis: a double blind, placebo, controlled randomised
475 clinical trial. Nutr Neurosci 2015;18:169-176.

476 41. Tarry-Adkins JL, Chen JH, Smith NS, Jones RH, Cherif H, Ozanne SE. Poor maternal
477 nutrition followed by accelerated postnatal growth leads to telomere shortening and increased
478 markers of cell senescence in rat islets. FASEB J 2009; 23: 1521-1528.

Table 1

Primer	Sequence (F)	Sequence (R)
Irs-1	TGGCAGTGAGGATGTGAAAC	CTTGGATGC TCCCCC TAGAT
p110-β	TGAGGTTGTGAGCACCTCTG	CTTTGTTGAAGGCTGCTGTG
Pkc-ζ	GGGTGGATGGGATCAAAATC	GGAGGACCTTGGCATAGCTT
Tnf-α	CCTCCTCTCTGCCATCAAGA	TGGAAGACTCCTCCCAGGTA
Tgfβ1	TGCCCTCTACAACCAACACA	CTTGCGACCCACGTAGTAGA
Leptin	AAGCCTCGCTCTACTCCACA	CATTCAGGGCTAAGGTCCAA
Mcp1	TGGACCAGAACCAAGTGAGA	TGCTGAAGTCCTTAGGGTTGA
Gp91^{phox}	CGAAGCCTTGGCTAAAACTCT	TCCTTGTTGAAGATGAAGTGGA
P22^{phox}	GTGAGCAGTGGACTCCCATT	GTAGGTGGCTGCTTGATGGT
P47^{phox}	TGTGACACCCTCTCACAGACA	GTCGCATTTTCCCTCCTTTA

Table 2: Effect of poor maternal nutrition and accelerated postnatal growth on body and organ weights in 3 month male rats.

Group	Body weight (g)	Epididymal fat pad weight (g)	Liver weight (g)
Control	483 ± 12	8.0 ± 0.5	17.0 ± 0.6
Recuperated	478 ± 8.8	9.0 ± 0.6 *	17.2 ± 0.4
Control CoQ	476 ± 12.3	8.2 ± 0.5	16.7 ± 0.6
Recuperated CoQ	482 ± 6.0	9.6 ± 0.5 *	18.0 ± 0.6

* $p<0.05$ (overall effect of maternal diet)

Table 3: Effect of poor maternal nutrition and accelerated postnatal growth on blood glucose, insulin and lipid profile in 3 month male rats.

Group	FPG (mmol/L)	Fasting serum insulin (mmol/L)	Total cholesterol (mmol/L)	FFAs (mmol/L)	Triglycerides (mmol/L)
Control (g)	5.6 ± 0.2	95 ± 26	2.3 ± 0.1	1052 ± 99	1.7 ± 0.3
Recuperated (g)	5.5 ± 0.2	49 ± 13	2.0 ± 0.1	990 ± 60	1.6 ± 0.1
Control CoQ (g)	5.4 ± 0.1	47 ± 13	2.1 ± 0.1	1051 ± 94	1.8 ± 0.2
Recuperated CoQ (g)	5.4 ± 0.1	47 ± 18	2.1 ± 0.2	976 ± 44	2.0 ± 0.4

Table 4: Effect of poor maternal nutrition and accelerated postnatal growth on insulin signaling molecule protein expression in 3 month male rats.

	Epididymal Fat		**Liver**		**Vastus lateralis**	
	Control	**Recup**	**Control**	**Recup**	**Control**	**Recup**
IR-β	100 ± 6	97 ± 9	100 ± 17	139 ± 17	100 ± 7	119 ± 10
p85-α	100 ± 7	101 ± 6	100 ± 11	111 ± 8	100 ± 7	112 ± 4
pAkt (ser473)	100 ± 20	130 ± 25	100 ± 10	110 ± 9	100 ± 17	141 ± 21
GLUT-4	100 ± 25	146 ± 21	-	-	100 ± 17	107.7 ± 21
Akt-1	100 ± 3	96 ± 3	100 ± 8	103 ± 6	100 ± 8	85 ± 11
Akt-2	100 ± 5	96 ± 3	100 ± 4	115 ± 7	100 ± 6	107 ± 6

Figure 1

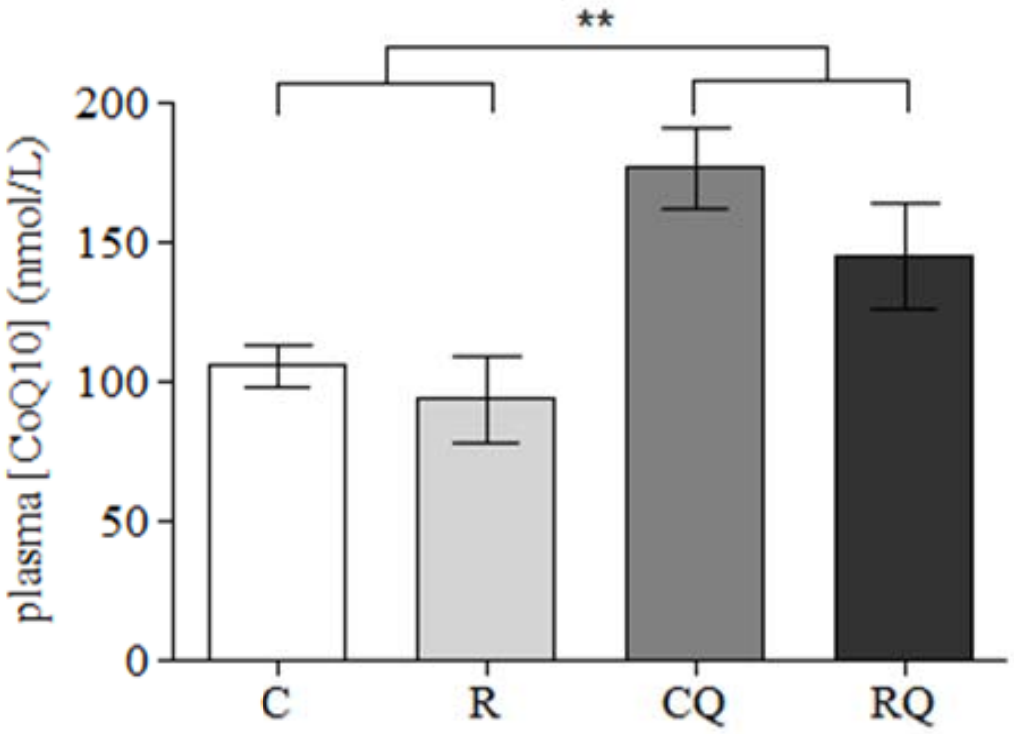


Figure 2

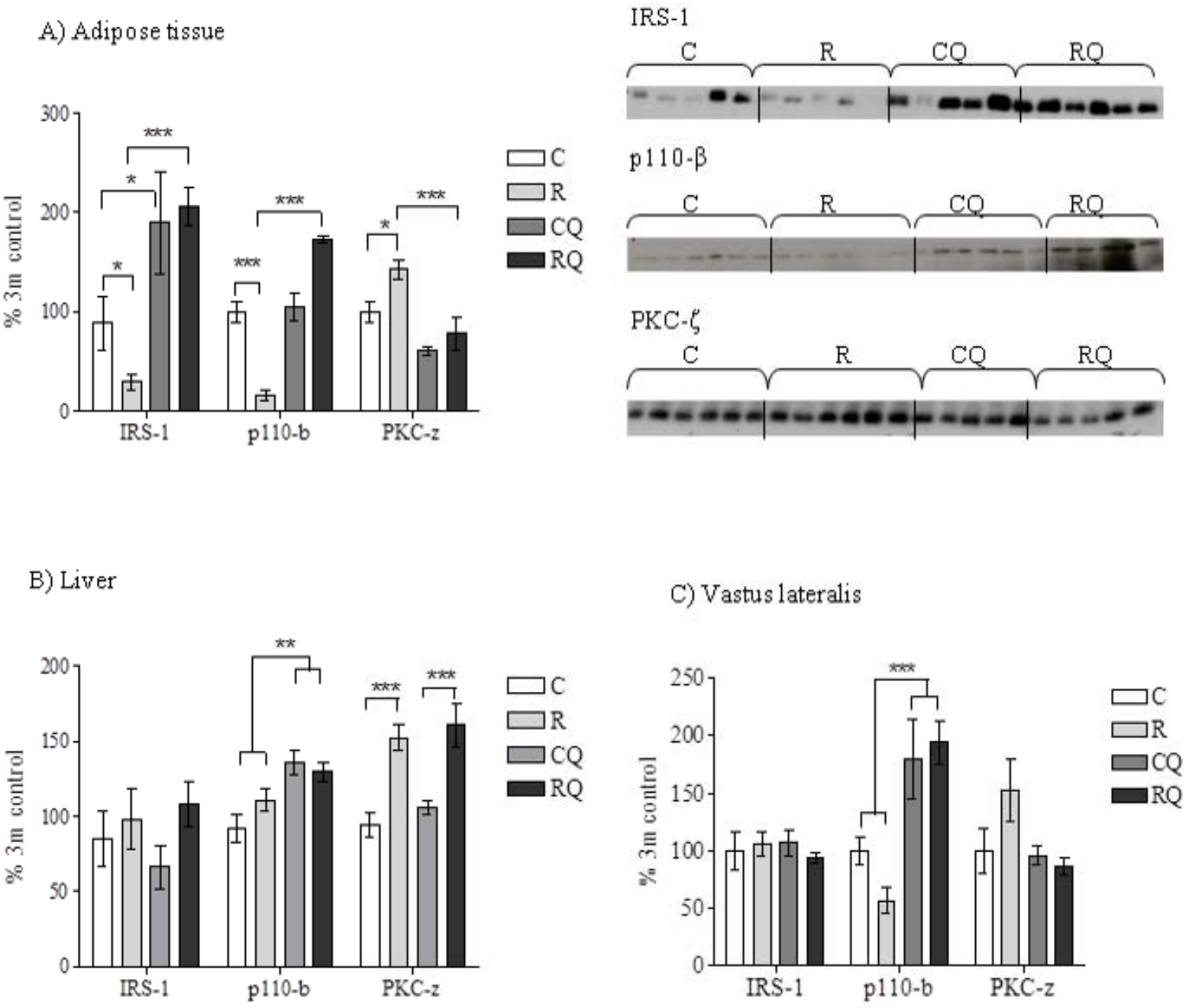

A) Adipose tissue
% 3m control
300
200
100
0
*
***
*
***
***
*
IRS-1
p110-b
PKC-z
C
R
CQ
RQ
IRS-1
C
R
CQ
RQ
p110-β
C
R
CQ
RQ
PKC-ζ
C
R
CQ
RQ
B) Liver
% 3m control
200
150
100
50
0
**
***
***
IRS-1
p110-b
PKC-z
C
R
CQ
RQ
C) Vastus lateralis
% 3m control
250
200
150
100
50
0
***
IRS-1
p110-b
PKC-z
C
R
CQ
RQ

Figure 3

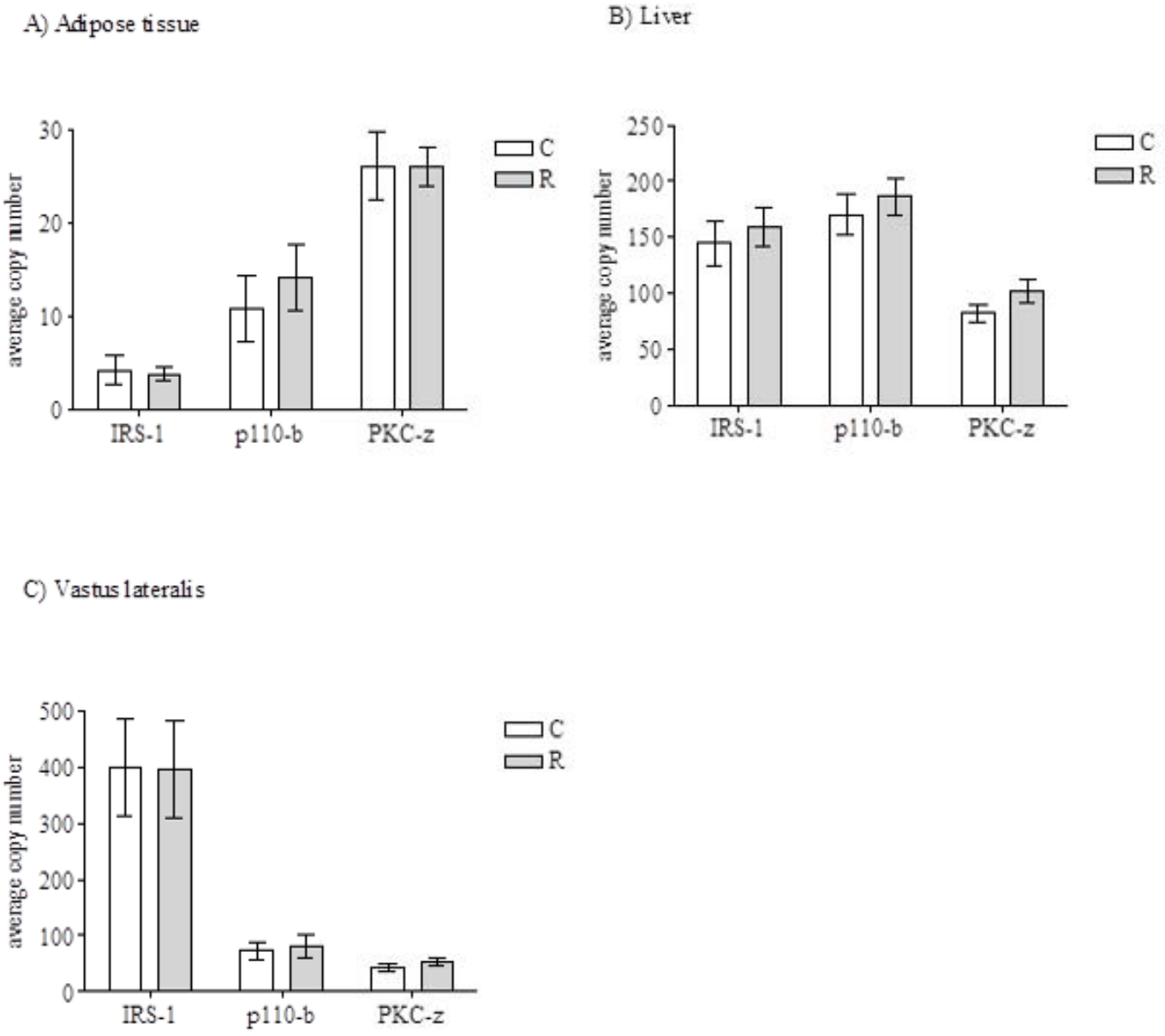
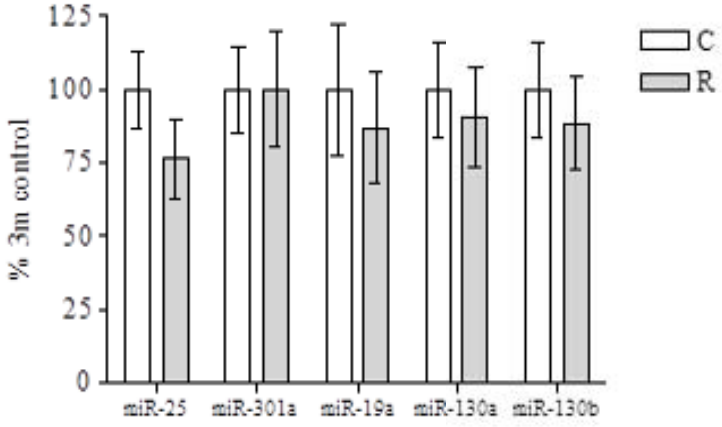

A) Adipose tissue
average copy number
30
20
10
0
IRS-1
p110-b
PKC-z
C
R
B) Liver
average copy number
250
200
150
100
50
0
IRS-1
p110-b
PKC-z
C
R
C) Vastus lateralis
average copy number
500
400
300
200
100
0
IRS-1
p110-b
PKC-z
C
R

Figure 4

A) miRNA targets to p110-β



B) miRNA targets to IRS-1

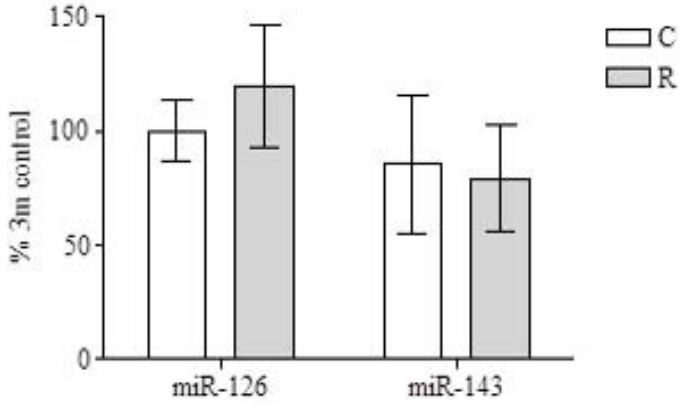
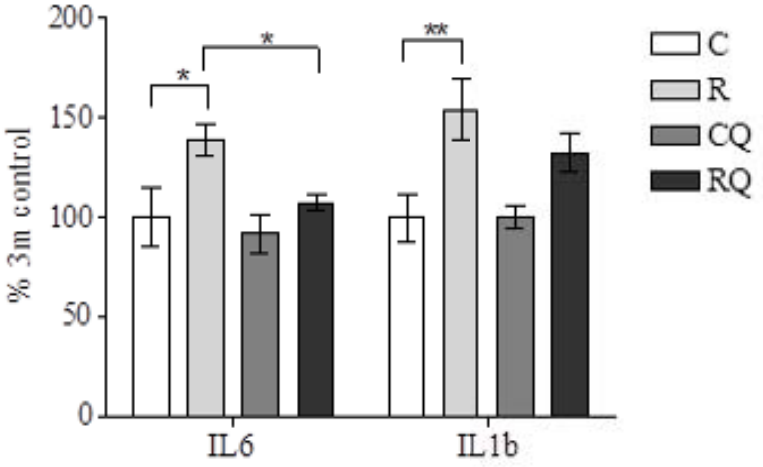
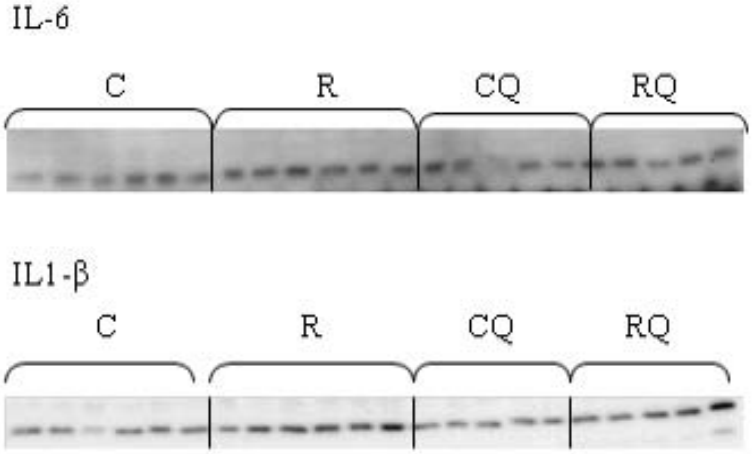


Figure 5

A) IL-6 and IL1β





B) Tnf-α, tgfβ1, leptin & mcp1

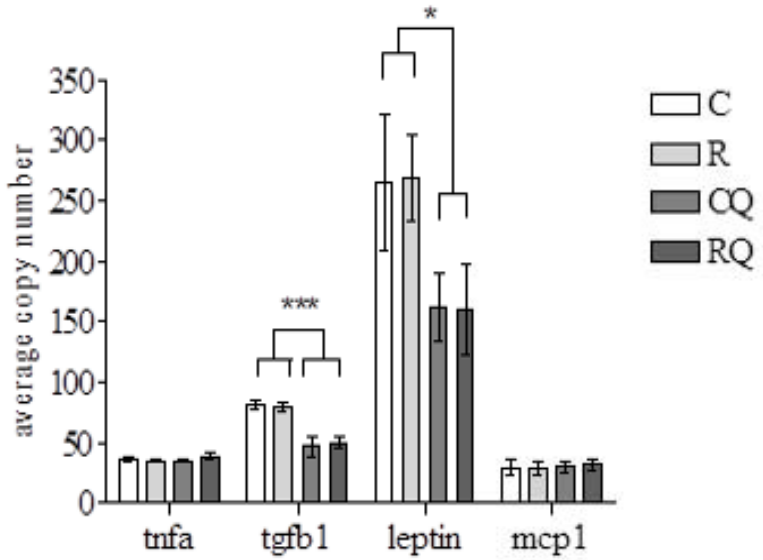
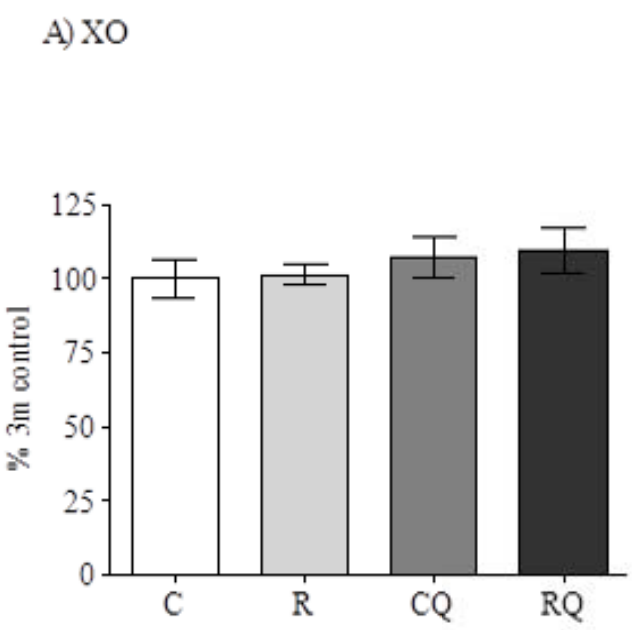
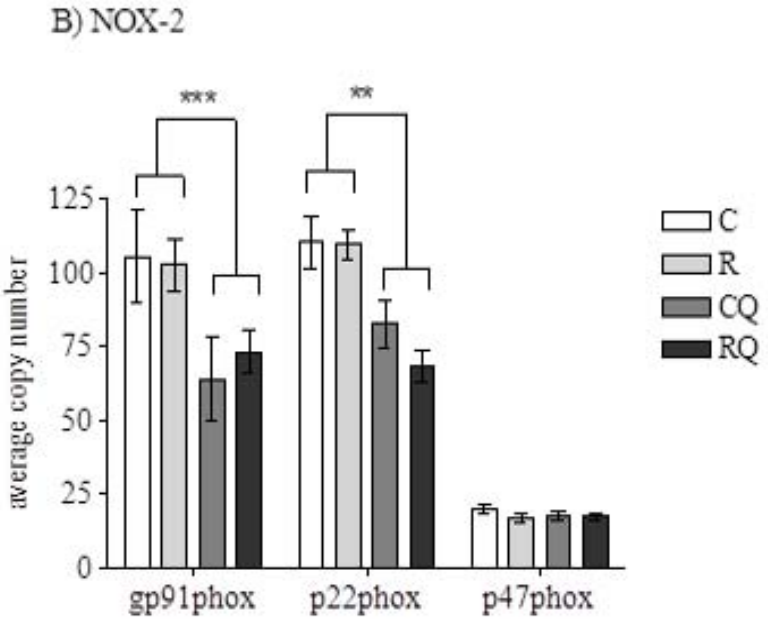
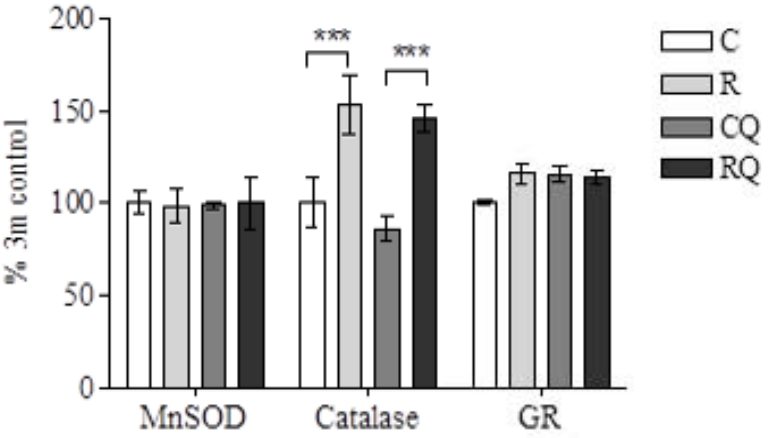


Figure 6


A) XO
% 3m control
125
100
75
50
25
0
C
R
CQ
RQ


B) NOX-2
***
**
average copy number
125
100
75
50
25
0
gp91phox
p22phox
p47phox
C
R
CQ
RQ


C) Antioxidant defense capacity
% 3m control
200
150
100
50
0
***
***
MnSOD
Catalase
GR
C
R
CQ
RQ