1 **Coenzyme Q$_{10}$ prevents insulin signaling dysregulation and inflammation prior to**

2 **development of insulin resistance in male offspring of a rat model of poor maternal**

3 **nutrition and accelerated postnatal growth**

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29 **ABSTRACT:**

30   Low birth-weight and rapid postnatal-growth increases the risk of developing insulin

31 resistance and type-2 diabetes in later life. However underlying mechanisms and potential

32 intervention strategies are poorly defined. Here we demonstrate that male Wistar rats exposed

33 to a low-protein diet *in-utero* that had a low birth weight but then underwent postnatal catch-

34 up growth (recuperated offspring) had reductions in the insulin signaling proteins p110-β [13

35 $\pm$ 6% of controls] ($p<0.001$) and IRS-1 [39 $\pm$ 10% of controls] ($p<0.05$) in adipose tissue.

36 These changes were not accompanied by any change in expression of the corresponding

37 mRNAs, suggesting post-transcriptional regulation. Recuperated animals displayed evidence

38 of a pro-inflammatory phenotype of their adipose tissue with increased interleukin-6 (IL-6)

39 [139 $\pm$ 8%], ($p<0.05$) and interleukin-1β (IL1-β) [154 $\pm$ 16%], ($p<0.05$) that may contribute to

40 the insulin signaling protein dysregulation. Post-weaning dietary supplementation of

41 recuperated animals with Coenzyme Q (CoQ$_{10}$) (1mg/kg of body weight/day) prevented the

42 programmed reduction in IRS-1 and p110-β and the programmed increased in IL-6. These

43 findings suggest that post-weaning CoQ$_{10}$ supplementation has anti-inflammatory properties

44 and can prevent programmed changes in insulin-signaling protein expression. We conclude

45 that CoQ$_{10}$ supplementation represents an attractive intervention strategy to prevent the

46 development of insulin resistance that results from suboptimal *in-utero* nutrition.

47

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

76 accelerated aging phenotype in the aorta (22) and heart (23) of rats that were born small and

77 underwent catch-up growth. CoQ exists in various isoforms which differ in concentration

78 between organisms. In humans, the most abundant form of CoQ is CoQ_{10} (as it contains 10

79 isoprenoid units attached to a benzoquinone ring); in rodents, however, CoQ_9 (containing 9

80 isoprenoid units) is the most abundant form. It is also known that rodents can convert dietary

81 CoQ_{10} into CoQ_9 (23). CoQ is the most abundant and potent antioxidant in the body (24) with

82 additional anti-inflammatory properties, including decreasing the production of pro-

83 inflammatory cytokines (25). Clinical trials have confirmed the safety of CoQ up to doses of

84 1200 mg/kg/day (26, 27).

85 This study therefore aimed to establish whether a postnatal dietary supplementation

86 of CoQ could alter any observed dysregulation of insulin signaling in rats exposed to a

87 suboptimal environment *in-utero* and postnatal catch-up growth and then to investigate the

88 potential underlying mechanisms, including oxidative stress status and inflammatory

89 phenotypes.

90

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, 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

146   RNA was extracted using a miRNeasy mini kit (Qiagen) following the manufacturers

147   instructions (22), a DNAse digestion step was performed in order to ensure no gDNA

148   contamination. RNA (1 ug) was used to synthesize cDNA using oligo-dT primers and M-MLV

149   reverse transcriptase (Promega, Southampton, Hants, UK). Gene expression was determined

150   using custom designed primers (Sigma) and SYBR Green reagents (Applied Biosystems).

151   (Primer sequences are described in Table 1).

152   Quantification of gene expression (expressed as average copy number) was

153   performed using the Step One Plus q-PCR machine (Applied Biosystems). Melting curve

154   analysis was performed to confirm absence of primer-dimers. Equal efficiency of the reverse

155   transcription of RNA from all groups was confirmed through quantification of expression of the

156   housekeeping gene *β-actin*. Expression did not differ between groups (effect of maternal diet

157   p=0.72; (control; 153 ± 32, recuperated; 134 ± 35 average copy number).

158

159   **microRNA (miRNA) analysis**

160   Putative miRNAs targeting the 3' untranslated regions (3'UTRs) of *p110β* and *IRS-1*

161   were identified using the miRanda/mirSVR 1.2 (Release 2010) and miRmap 3 (Release 1.1)

162   prediction algorithms, respectively. Candidates were ranked according to the strength of the

163   predicted interaction, high conservation across species and target site position along the

164   mRNA (higher scores for proximal and distal locations). The final selection of the most

165   highly ranked candidates to assess was based on common target sites within both the *p110β*

166   and *IRS-1* 3'UTRs as well as information regarding their expression in white adipose tissue.

167   RNA was extracted using a Direct-zol™ RNA MiniPrep kit (Zymo Research), in order to

168   retain RNA species with a minimal length of 17 nucleotides. RNA purity and concentration

169   was determined by spectrophotometric analysis on a NanoDrop ND-1000. RNA integrity was

170   confirmed by denaturing agarose gel electrophoresis.

171

172   **Statistical Analysis**

173   Data were analyzed using a 2-way ANOVA with maternal diet and CoQ

174   supplementation as the independent variables. Data are represented as mean ± S.E.M. A

175   value of $p<0.05$ was considered statistically significant. All statistical analyses were

176   performed using Statistica 7 software (Statsoft Inc) except for miRNA analysis where

177   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

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205   molecules including IRβ, p85α, Akt-1, Akt-2, pAkt$^{(Ser473)}$, and GLUT-4 were unaffected by

206   maternal diet in the epididymal fat, liver and vastus lateralis muscle (Table 4).

207   The programmed reduction in adipose tissue IRS-1 and p110-β in the absence of any

208   differences in the corresponding mRNAs suggested that this effect was mediated by a post-

209   transcriptional mechanism. However, we saw no effects of maternal diet on the five miRNAs

210   predicted to regulate *p110-β* (miR-25, miR-301a, miR-19a, miR-130a and miR-130b) or the

211   two predicted to regulate IRS-1 (miR-126 and miR-143), (Figures 4A and B).

212   **b) Effect of CoQ supplementation:** CoQ supplementation significantly increased both IRS-1

213   and p110-β protein levels in recuperated offspring epididymal fat. In control offspring, CoQ

214   supplementation had no effect on p110-β levels; however supplementation increased IRS-1

215   protein expression (Figure 2A). CoQ supplementation increased ($p < 0.001$) p110-β protein

216   expression in both liver and VL skeletal muscle (Figures 2B and C). CoQ supplementation

217   significantly ($p < 0.001$) decreased PKC-ζ protein levels in recuperated adipose tissue

218   compared to the effect on control offspring (Figure 2A); however, PKC-ζ expression

219   remained similar in the liver and vastus lateralis muscle (Figures 2B and C).

220   Cytokine analysis

221   Adipose tissue protein expression of IL-6 ($p < 0.05$) and IL-1β ($p < 0.01$) were increased

222   in recuperated offspring (Figure 5A). CoQ supplementation significantly ($p < 0.05$) reduced IL-

223   6 levels back to that of the controls, however, IL-1β levels were unchanged by CoQ

224   supplementation (Figure 5 A). mRNA levels of *Tgfb1* and *Lep* were unaltered by maternal

225   diet; however, CoQ supplementation reduced *Tgf-β1* ($p < 0.001$) and *Lep* ($p < 0.05$) levels

226   (Figure 5B). *Tnf-α* and *Mcp-1* gene expression were similar between all groups (Figure 5B).

227   There was no significant effect of maternal diet or CoQ_{10} supplementation on adiponectin

228   (*adipoq)* mRNA in epididymal fat (control: $100 \pm 26.6\%$; recuperated: $107 \pm 18\%$; control

229   CoQ: $91 \pm 32\%$; recuperated CoQ: $73 \pm 9\%$).

<u>Reactive oxygen species (ROS) and antioxidant defense capacity</u>

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

271 insulin signaling protein expression. However, uptake of CoQ into most tissues is thought to

272 be low (35) and a systemic effect is likely to be involved.

273 Inflammation is an important factor in the development of insulin resistance via

274 inhibition of insulin signaling through activation of IKK-β and JNK pathways (36). IL-6, IL-

275 1β, tumor necrosis factor-α (TNF-α), monochemoattractant protein-1 (MCP-1), transforming

276 factor-β1 (TGF-β1) and leptin are major cytokines and chemokines that can be secreted by

277 dysfunctional adipocytes or infiltrated adipose tissue macrophages. These factors play a

278 pivotal role in adipose tissue-induced low-grade systemic inflammation and/or obesity. In the

279 current study, we demonstrated increased protein expression of IL-6 and IL-1β in the

280 epididymal fat pads of recuperated offspring. IL-6 is known to inhibit protein and mRNA

281 expression of IRS-1 (37), suggesting that the increased IL-6 levels could, at least in part,

282 explain the observed reduction in IRS-1 protein levels in recuperated animals. Furthermore,

283 IL-1β is a key factor in mediating macrophage-induced insulin resistance in human adipocytes

284 (38). However Mcp-1 expression (a key chemokine that regulates migration and infiltration of

285 monocytes and macrophages) was unaltered between groups, suggesting that any macrophage

286 infiltration in recuperated adipose tissue could be via an Mcp-1 independent mechanism.

287 Interestingly, this pro-inflammatory phenotype occurred in the absence of obesity, suggesting

288 that maternal suboptimal nutrition is the driving factor for this effect in a manner that is

289 independent of offspring adiposity. CoQ supplementation significantly reduced IL-6 protein

290 and *Tgf-β1* and *Lep* mRNA levels, which is in agreement with findings in cardiac tissue (25),

291 and CoQ is known to have anti-inflammatory properties in mouse liver (39) and human

292 plasma (40) however, to our knowledge this is the first time that CoQ has been demonstrated

293 to have anti-inflammatory properties in adipose tissue. This highlights the function of CoQ as

294 an important anti-inflammatory molecule and may partially explain some of our reported

295 'insulin sensitizing' effects of CoQ.

296 Over-production of reactive oxygen species (ROS) is well-known to contribute to the

297 development of insulin resistance. In the current study, however, no evidence of an oxidative

298 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:

320 Figure 1 - The effect of *in utero* protein restriction, accelerated postnatal growth and CoQ

321 supplementation upon plasma CoQ levels in 3-month-old male rats. Results are expressed as

322 mean ± S.E.M. * $p < 0.01$ (C and R versus CQ and RQ). C = control; CQ = control CoQ; R =

323 recuperated; RQ = recuperated CoQ. N = 10 per group.

324 Figure 2 - The effect of *in utero* protein restriction, accelerated postnatal growth and CoQ

325 supplementation upon protein expression of insulin signaling molecules in A) epididymal

326 adipose tissue, B) liver and C) VL skeletal muscle in 3-month-old male rats. Results are

327 expressed as mean ± S.E.M. * $p < 0.01$ and *** $p < 0.001$ (C versus R) and * $p < 0.01$ and ***

328 $p < 0.001$ (R vs RQ). C = control; CQ = control CoQ; R = recuperated and RQ = recuperated

329 CoQ. N = 5 - 6 per group.

330 Figure 3 - The effect of *in utero* protein restriction and accelerated postnatal growth upon

331 gene expression of insulin signaling molecules in A) epididymal adipose tissue, B) liver and

332 C) VL skeletal muscle in 3-month-old male rats. Results are expressed as mean ± S.E.M. N =

333 8 per group.

334 Figure 4 - The effect of *in utero* protein restriction and accelerated postnatal growth upon

335 miRNA targets to A) *p110β* and B) *IRS-1*. Results are expressed as mean ± S.E.M. N = 10 per

336 group.

337 Figure 5 - The effect of *in utero* protein restriction, accelerated postnatal growth and CoQ

338 supplementation upon cytokine protein and gene expression in epididymal adipose tissue in 3-

339 month-male rats. Results are expressed as mean ± S.E.M. * $p < 0.05$ (C versus R) and * $p < 0.05$

340 (R vs RQ). C = control; CQ = control CoQ; R = recuperated and RQ = recuperated CoQ. N =

341 5 - 6 per group.

342 Figure 6 - The effect of *in utero* protein restriction, accelerated postnatal growth and CoQ

343 supplementation upon A) and B) sources of ROS and C) antioxidant defense capacity in 3-

344 month-old male rat epididymal adipose tissue. Results are expressed as mean ± S.E.M. *

345 p<0.05 (C and R versus CQ and RQ). C = control; CQ = control CoQ; R = recuperated and

346 RQ = recuperated CoQ. N = 6 per group.

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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}	TGTGACACCTCTCACAGACA	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
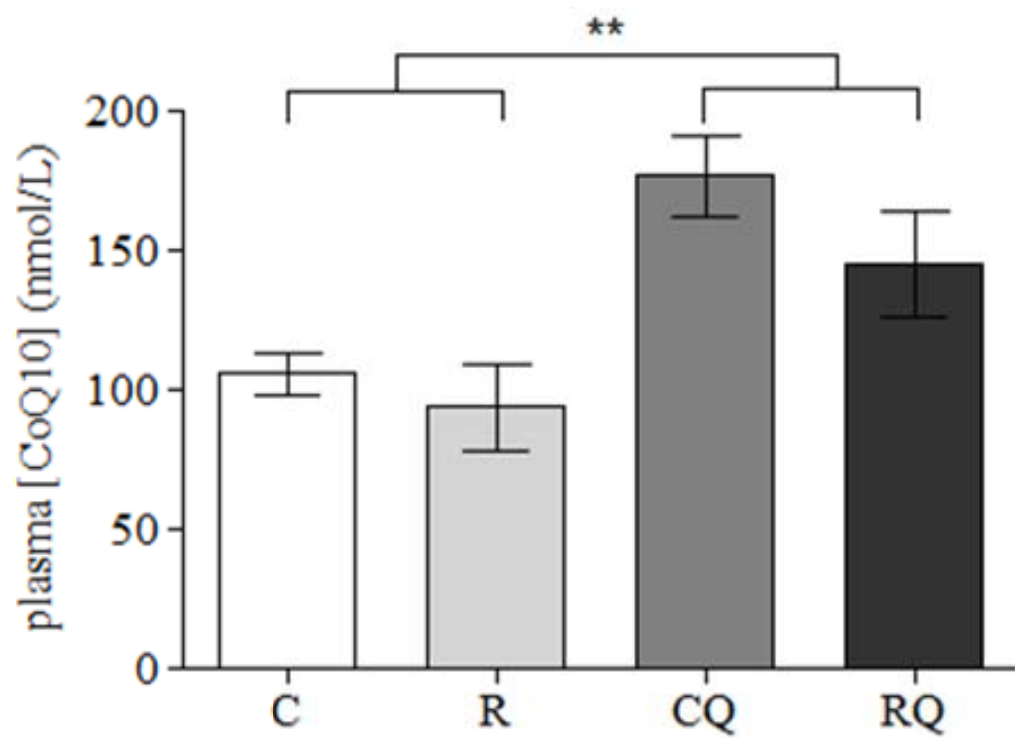
Figure 2

Figure 3

A) Adipose tissue
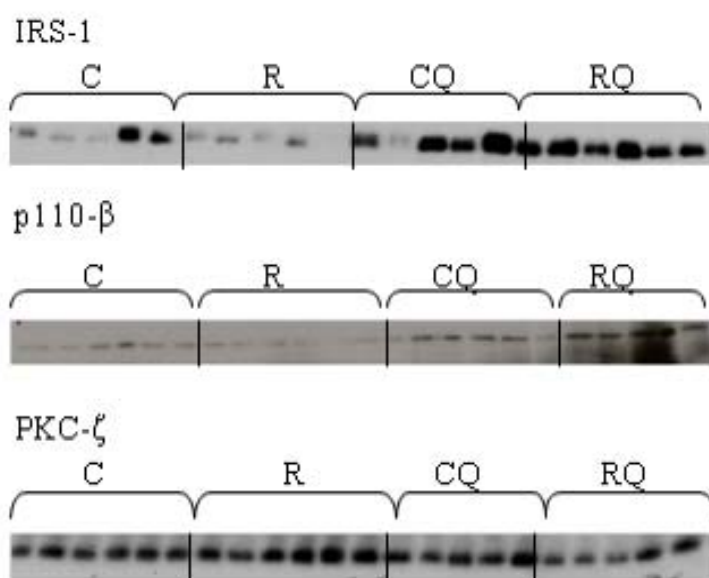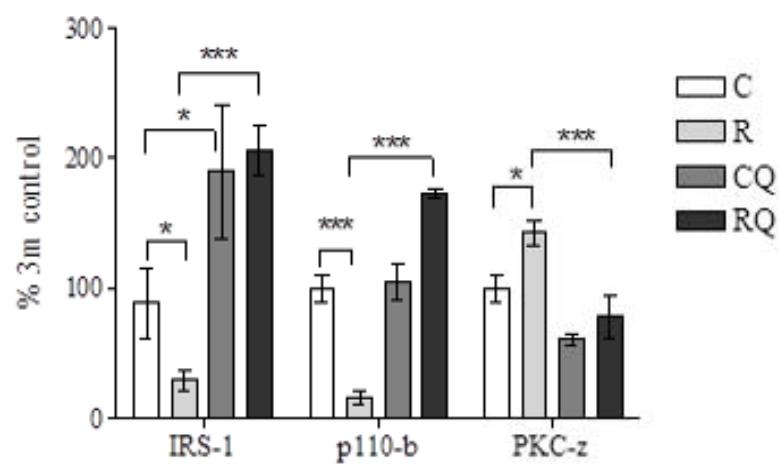
B) Liver
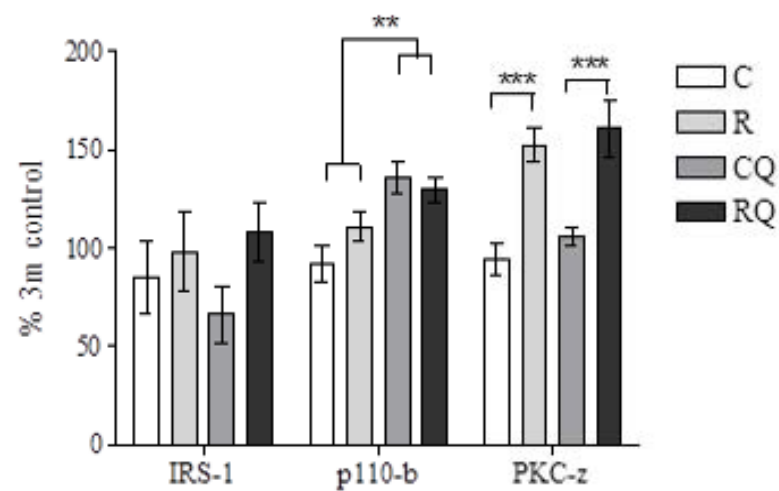
C) Vastus lateralis
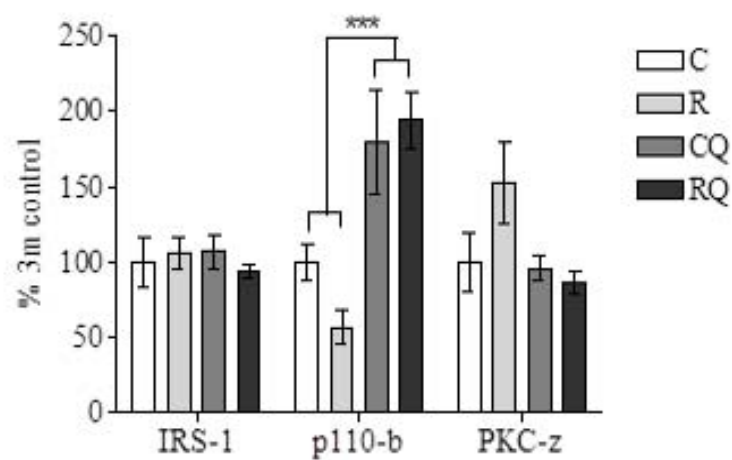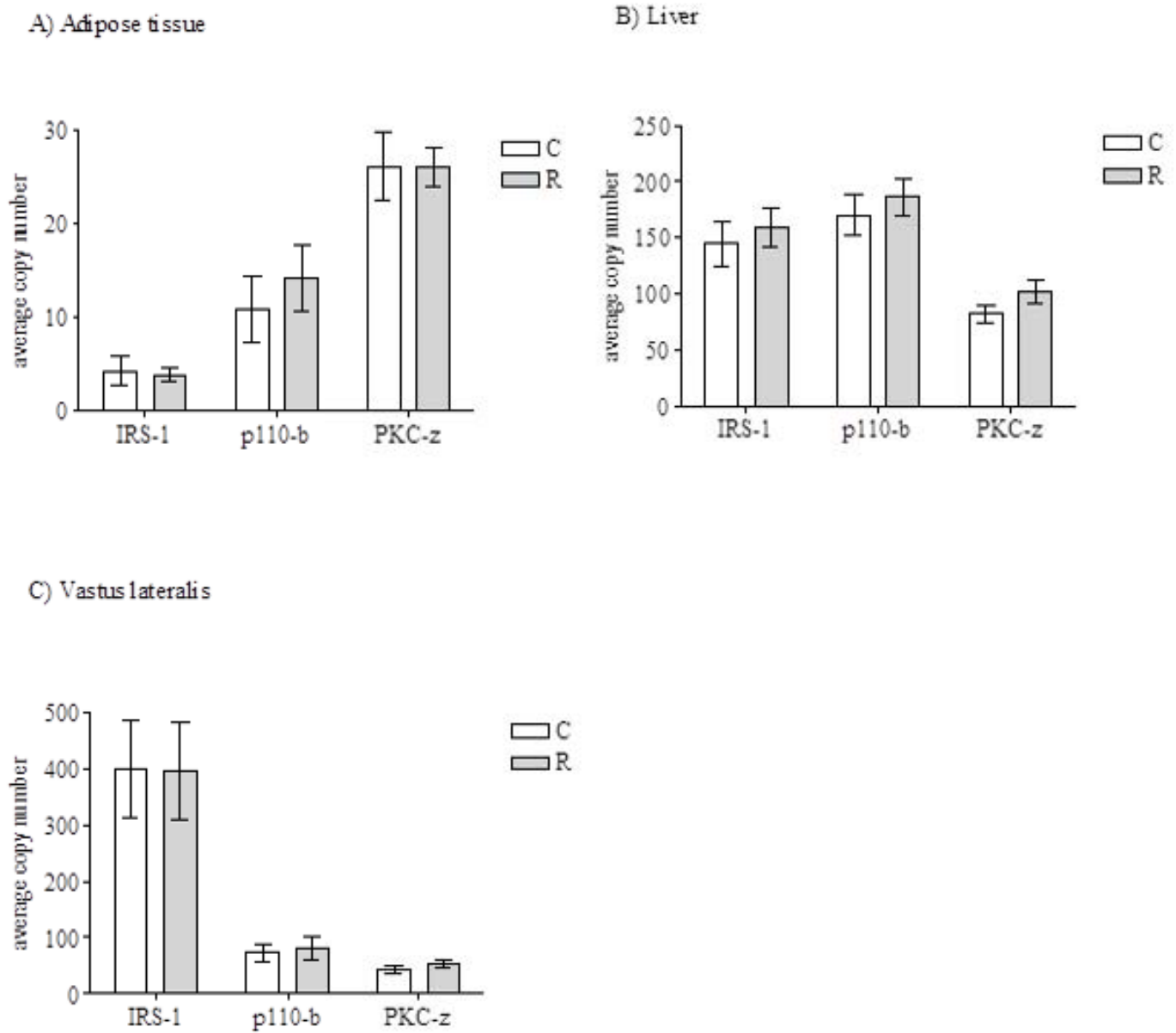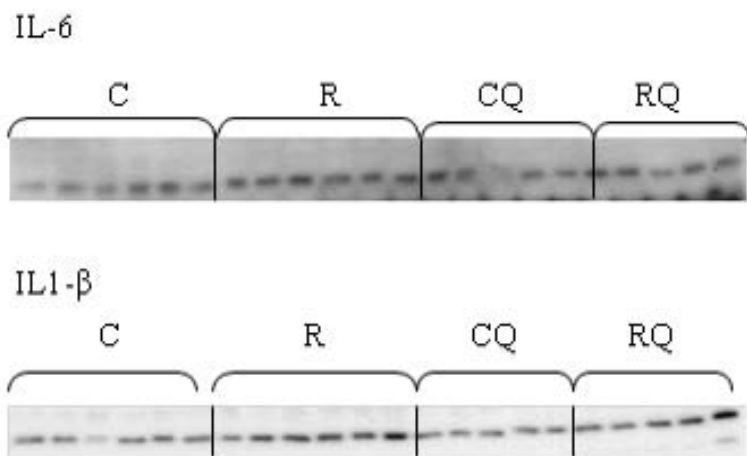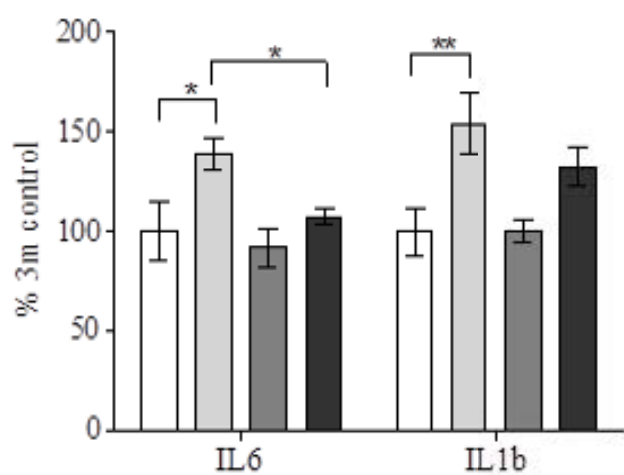
Figure 4

A) miRNA targets to p110-β
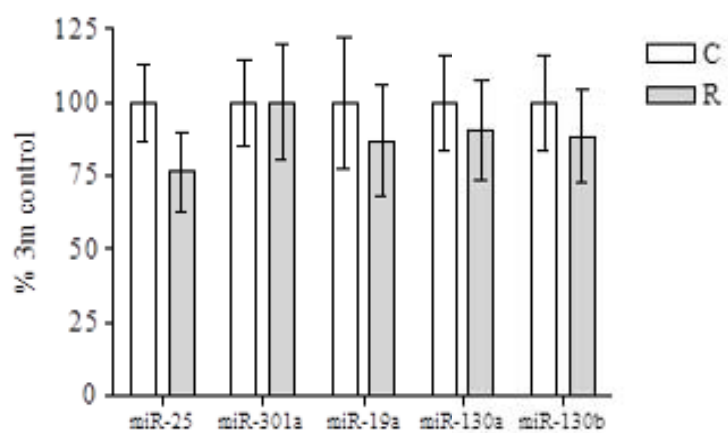
B) miRNA targets to IRS-1
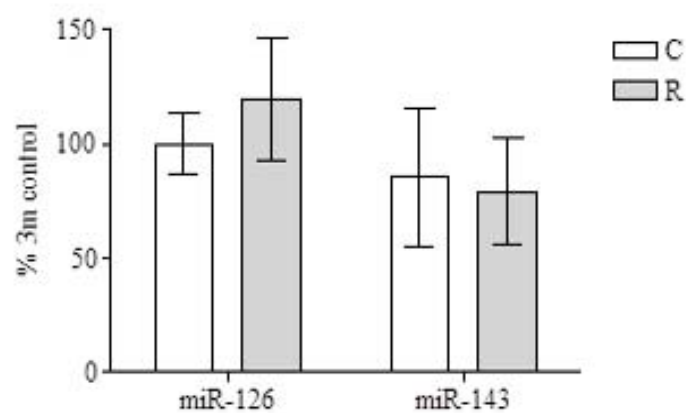
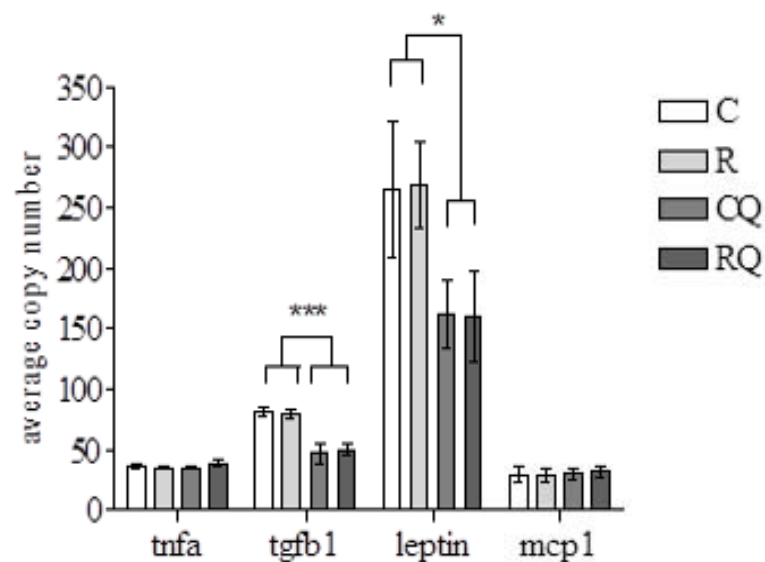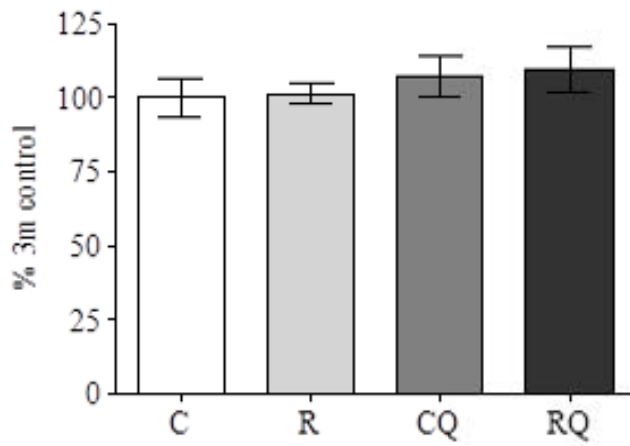Figure 5

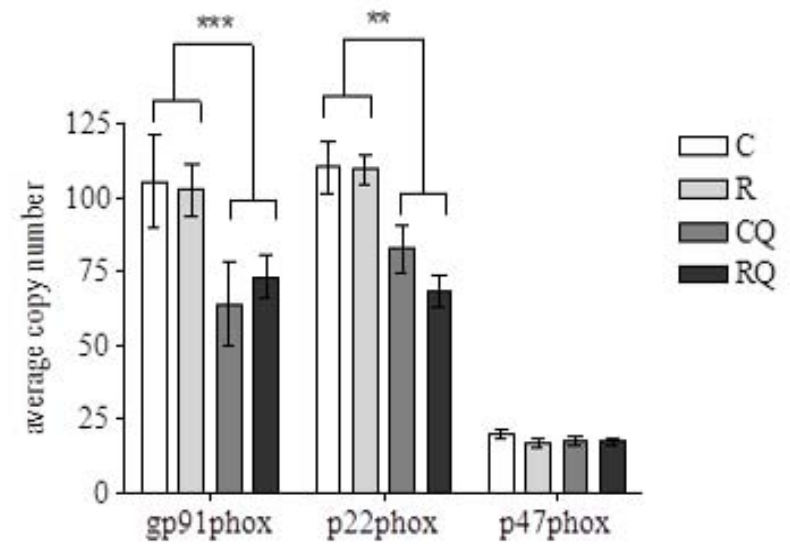A) IL-6 and IL1β

B) Tnf-α, tgfβ1, leptin & mcp1

Figure 6

A) XO

B) NOX-2

C) Antioxidant defense capacity