

DISSERTATION

EFFECT OF AGING ON GENE EXPRESSION BY GRANULOSA CELLS FROM MARES

Submitted by

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## ABSTRACT

### EFFECT OF AGING ON GENE EXPRESSION BY GRANULOSA CELLS FROM MARES

Changes in gene expression in granulosa cells from mares may result from aging or development of metabolic disease as well as from other causes. We used qRT-PCR to quantify expression of equine insulin receptor (*IR*), insulin-like growth factor receptor (*IGF-1R*), glucose transporter type 4 (*GLUT4*) and adenosine monophosphate-activated protein kinase (*AMPK*) subunit genes in granulosa cells from young (4-9 years), middle-aged (10-16 years) and older (>16 years) mares. Granulosa cells were isolated following follicular aspiration and incubated in the presence or absence of 10% fetal bovine serum (FBS) and 30mM glucose for 24 hrs. Cells were then treated for 1 hr with insulin (100nM), IGF-1 (10nM), epidermal growth factor (EGF; 1 $\mu$ M), progesterone (P4; 100 nM) or human chorionic gonadotropin (hCG; 100 nM).  $\Delta$ Ct, where  $\Delta$ ct is equal to the CT value for the gene of interest minus the CT value for a house keeping gene, values for *IGF-1R* or *IR* expression did not differ significantly for any age group. However, there were statistically significant differences in the ratio of  $\Delta$ Ct for *IR* relative to  $\Delta$ Ct for *IGF-1R* for individual young, middle-aged and old mares. Young animals expressed increased *IGF-1R* relative to *IR* after pre-incubation of cells in either – fetal bovine serum FBS/30mM glucose or + FBS/30mM glucose media followed by treatment with insulin, IGF-1, or EGF. Conversely, middle-aged animals expressed increased *IR* relative to *IGF-1R* following preincubation in both medium and following treatment with insulin or IGF-1. Finally, older animals expressed approximately equal amounts of *IR* relative to *IGF-1R* (-FBS/-30mM glucose) or increased *IR* relative to *IGF-1R*.

When cells were pre-incubated in +FBS/+30mM glucose medium and treated with insulin or IGF-1, the ratio of IR expression relative to IGF1R expression changed and there was increased *IGF-1R* relative to *IR*. Changes in the relative numbers of IR and IGF-1R monomers expressed from *IR* and *IGF-1R* genes may affect the particular receptor dimers assembled from these monomers. In evaluating fold change  $2^{-\Delta\Delta Ct}$  in gene expression, we observed increases in *IGF-1R* expression (+FBS/+30mM glucose;  $p < 0.03$ ), *GLUT4* expression (+FBS/+30mM glucose;  $p < 0.05$ ) and *AMPK $\alpha$ 2* expression (-FBS/-30mM glucose;  $p < 0.03$ ) in older mares relative to young animals. Together, these results indicate that young animals differ from older animals in several ways. Young animals maintain stable ratios of  $\Delta Ct$  for *IGF-1R* to  $\Delta Ct$  for *IR* and these ratios are not affected by treatment with insulin, IGF-1 or EGF. Thus, it is likely that young animals maintain higher levels of IGF-1R homodimers and hybrid receptors formed from IGF-1R and insulin receptor monomers and that receptor numbers, including insulin receptors, remain stable, particularly when circulating glucose and insulin levels are high. Older animals, on the other hand, are more labile with respect to  $\Delta Ct$  for *IGF-1R* relative to  $\Delta Ct$  for *IR*. Exposure of granulosa cells pre-incubated in +FBS/+30mM glucose medium to high levels of glucose, insulin, IGF-1 or EGF demonstrated increased *IGF-1R* expression. This may decrease numbers of insulin-responsive insulin homodimers, increasing hybrid receptors and IGF-1R homodimers and thus decreasing insulin responsiveness and insulin effects on metabolism. Moreover, when there are significant effects on expression of *IR*, *IGF1R* or *GLUT4* in older animals, there are accompanying increases in expression of some *AMPK* subunit genes in older animals indicating additional effects of aging on overall metabolism.

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DEDICATION

*To MY FAMILY*

*Nadya. M*

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## CHAPTER 1: BACKGROUND AND SIGNIFICANCE

### 1.1: Introduction

The equine follicle and human follicle are finely-tuned microenvironments in which cell-cell communication and exchange of soluble factors are essential to development of the dominant follicle and production of a fertilizable oocyte (1). The effects of aging on follicular maturation and fertility in mares and women have led to suggestions that the equine model may be an appropriate model for studies of reproductive aging in women (2). As in women, mare gonadotropins are secreted by the anterior pituitary gland and bind their respective receptors on granulosa cells in the follicle. Activation of these receptors is necessary for follicle development and production of cell-secreted factors including epidermal growth factor (EGF)-like hormones that act locally on the oocyte or on cumulus cells associated with the oocyte (2). Although gonadotropin responsiveness by bovine granulosa cells is enhanced in the presence of insulin and insulin like growth factor-1 (IGF-1) (3), very high concentrations of IGF-1 decrease embryo viability and increase apoptosis (4). In addition, changes in the metabolic status of mares (and women) including reduced insulin sensitivity affect fertility at any age (5). In mares, insulin insensitivity produces aberrant reproductive cycles (6) and is often associated with obesity. In humans, insulin resistance and obesity are correlated with polycystic ovarian syndrome (PCOS), a major cause of infertility in women (7).

Because metabolic disorders are a common concern in aging mares, our goal in these studies was to examine the relationship between gene expression for insulin receptor (IR) and insulin-like growth factor-1 receptor (IGF-1R) and cellular metabolism in granulosa cells. These

studies used granulosa cells from young mares (4-9 years), middle aged mares (10-16 years) and older animals (>16years) and were conducted in medium containing either high levels of glucose (30 mM) and 10% fetal bovine serum as a source of growth factors (+FBS/+30 mM glucose) or in medium lacking additional glucose and FBS (-FBS/-30 mM glucose). We used real-time reverse transcription polymerase chain reaction (RT-PCR) to quantify expression of equine IR and IGF-1R genes in granulosa cells in response to epidermal growth hormone (EGF), IGF-1, insulin, progesterone (P4) and human chorionic gonadotropin (hCG). We also examined effects of these hormones on the expression of the glucose transporter type 4 (GLUT4) gene which is up-regulated by insulin, and on the levels of adenosine monophosphate-activated protein kinase (AMPK) isoform genes which reflect the metabolic activity of granulosa cells.

## **1. 2: Selection of the dominant follicle and hormonal effects on follicle function**

Dominant follicle growth in an ovulatory phase is associated with follicular waves and processes that lead to continual growth of the dominant follicle and regression of other subordinate follicles (8,9). The structure of the follicle and the relationship between granulosa cells and the oocyte are shown in **Figure 1.1**. In women, ovulatory waves resulting from increasing follicle stimulating hormone (FSH) reach their peak when the largest follicle in size is about 13 mm in diameter. Following the FSH surge, there is a decrease in FSH which is linked to increased levels of inhibin. Circulating estradiol begin to increase about two days after the FSH peak and one day prior to the beginning of deviation. Follicle deviation occurs when one or two dominant follicles are apparent and where the dominant follicle is  $\geq 28$  mm in diameter (10,11). One day before the beginning of diameter deviation, the intra-follicular concentrations of estradiol, IGF-1, inhibin A and activin A increase differentially in what will become the dominant follicle. This follicle, the

dominant follicle, can grow to greater than 28 mm in mares. The remaining follicles then undergo atresia (12).

The appearance of follicular deviation and a dominant follicle is influenced by the IGF-1 and are dose dependent. Injection of a physiological dose of IGF-1 into the second largest follicle of the mare prior to deviation increased the concentration of inhibin A and activin A, but not estradiol within 24 hours post injection (PI). The 500- and 250- $\mu$ g doses stimulated follicle growth and ovulation growth. Injection of IGF-1 binding protein into the dominant follicle at the expected beginning of deviation decreased the follicle size within 24 hours (13).

Members of the IGF-1 super family in mammals are reproductively important hormones. The IGF-1 super family in mammals includes IGF-1 and IGF-2 and their receptors. In both equine and bovine species, accumulation of IGF-1 occurs in the dominant follicle and in larger amounts than in the regressing follicles. IGF-1 in the dominant follicle leads to secretion of activin A, inhibin A, and vascular endothelial growth factor (VEGF). These local IGF-1 effects are probably involved, at least in part, for selection of a dominant follicle in the mare (14). Ginther et al. demonstrated that IGF-1 and its receptor played a critical role in expression of follicular-fluid factors leading to selection and growth of the dominant follicle in mares (15). IGF-1 acts with FSH in granulosa cells to stimulate gonadal steroid production. Knockout of IGF-1 or FSH leads to infertility and hypogonadism (16). Thus IGF-1, in addition to FSH and LH, may play an important role in regulation of estradiol production in the human ovary (17).

EGF and EGF-related proteins as growth promoting factors in the follicle by providing autocrine and/or paracrine signals that facilitate the LH stimulus. The EGF signaling system consists of EGF-related ligands and tyrosine kinase receptors called collectively described as the epidermal growth factor receptor (EGFR) family and also known as ErbB receptors.

Amphiregulin (AR), epiregulin (EPI), and beta cellulin (BTC), each with EGF-like motifs, are capable of binding the EGF receptor (18,19). EGF-like growth factors appear to function as mediators of LH action in the dominant follicle. LH regulates mRNA and protein synthesis and LH activation of the LH receptor leads to regulation of EGF-like growth factors at the transcriptional level and processing of ligand precursors. Soluble EGF-like growth factors then act as autocrine and paracrine hormones to activate EGFR and to increase metabolic activity within target cells. One activated enzyme, AMPK, which is discussed later in this section, is necessary for LH- and EGF-mediated cumulus expansion and oocyte maturation.

In addition to follicular growth, the oocyte and granulosa cells develop intimate intercellular connections (20,21). Both the oocyte and granulosa cells have cytoplasmic projections and microvilli that interdigitate with each other to create a large surface area for diffusion (22). In addition, some of the follicle cell microvilli and cytoplasmic projections physically penetrate deeply into the oocyte via invagination of the oocyte plasma membrane, occasionally reaching close to the nuclear membrane to control local FSH levels (11).

### **1.3: Aging effects on follicular development**

Female reproductive organs show signs of aging earlier than tissues or organs in the body. In women, fertility begins to decrease in the late 30s. However, an ovulatory cycle continues to produce ova until menopause at a mean age of 50 years (23). In most women, ovarian function diminishes between 50 and 55 years of age and the serum FSH concentration becomes higher than that seen in younger women (24). Hormone levels of LH, FSH, estradiol (E2), and progesterone (P) also change with increasing age and the inter-menstrual interval increases. In women, cycle length is 28 days between the ages 25 and 40 years and the cycle length gradually

decreases until menopause (24). Changes in cycle length appear to be due to changes in follicular maturation. A shorter follicular phase in premenopausal women is accompanied by increased levels of FSH and decreased estradiol levels throughout the cycle. With aging, short cycles are interspersed with long cycles where there is no evidence of ovulation. Aging follicles have fewer granulosa cells and these cells are less responsive to LH (25).

Aging in mares also affects development of the dominant follicle. In mares over 20 years of age, development of a dominant follicle and delayed emergence of subordinate follicles is a common occurrence (12). Major anovulatory waves precede the wave that initiates ovulation. Similarly to women, the major anovulation wave has been reported to occur in horses and other monovular species. Mares are also reported to have anovulatory and ovulatory seasons with the transition between these seasons occurring in the spring (13).

Changes in the ovarian cycle in both humans and mares are associated with decreased pregnancy rates. Healy et al. (1994) showed that fecundity in middle-aged women decreased about 50% as women aged from 25 to 35 years (26). In Amish women who did not use contraceptives, the median age for the last birth was 38.5 years and only about 7% of births occurred in women between 40 and 44 years of age. The age of women at last delivery was 10 years before the age at menopause (27). In mares, overall fertility in mares begins to decrease in at approximately 12 years of age and then declines linearly until the late 20s. Madill (2002) showed that the conception rate per cycle was 57% in younger mares (2-11 years) and 31% in older mares (>14 years) while foaling rates per cycle were 51% and 13%, respectively, and foaling rates per season were 82% and 48%, respectively (28).

#### **1.4: Metabolic disorders can accompany aging and affect follicular development**

In addition to aging, fertility in women and mares may be affected by metabolic disorders. Obesity modifies the aging process through effects on energy homeostatic mechanisms. Obesity may contribute indirectly to the development of age-related diseases such as diabetes mellitus and atherosclerosis (29). Aging is one of the most common causes of impaired leptin sensitivity (30). Leptin is a hormone secreted by adipocytes that can cause suppression of appetite.

Changes in fat distribution associated with the onset of metabolic disorders may cause the accumulation of lipids in the adipose tissue, particularly in the abdomen. In women, menopause is associated with an increase in intra-abdominal fat, perhaps as a result of loss of ovarian function. Ovariectomy in rats resulted in weight gain and increased intra-abdominal fat deposition. In knock-out mice that have aromatase defect or loss of estrogen receptor  $\alpha$ , obesity and associated metabolic disorders are common (31,32). Menopause does not seem to affect androgen production as adrenal glands continue to secrete androgen precursors such as dehydroepiandrosterone-sulfate (DHEA-S) and androstenedione (33).

#### **1.5: Cellular metabolism, AMP kinase activity and aging**

AMP-activated protein (AMP) kinases are active during decreases in overall cellular energy and increases ATP production while decreasing energy-utilization in anabolic processes (34,35). AMP kinase (AMPK) is a heterotrimeric enzyme that is evolutionarily conserved from yeast to mammals and functions to maintain cellular and whole body energy homeostasis.

The 5' AMPK-activated protein kinase is serine/threonine protein kinase (34,36). There are two genes encoding isoforms of both  $\alpha$  and  $\beta$  subunits ( $\alpha$ 1,  $\alpha$ 2,  $\beta$ 1 and  $\beta$ 2) and three genes encoding isoforms  $\gamma$  subunit  $\gamma$ 1,  $\gamma$ 2 and  $\gamma$ 3. The AMPK holoenzyme consists of one catalytic

subunit  $\alpha$  and two functionally and structurally different regulatory subunits ( $\beta$ ,  $\gamma$ ). Two isoforms have been identified for  $\alpha$ , and  $\beta$  subunits  $\alpha_1$ ,  $\alpha_2$ ,  $\beta_1$ ,  $\beta_2$ , and three isoforms have been identified for the  $\gamma$ -subunits,  $\gamma_1$ ,  $\gamma_2$ , and  $\gamma_3$  (37). AMP binds AMPK via two cystathionine beta synthase (CBS) domains on the  $\gamma$  subunit and activates AMPK by an allosteric mechanism (**Figure 1.2**). AMP makes AMPK a better substrate for upstream kinase and poorer substrate for phosphatases (31,38,39) perhaps because binding of AMP to  $\gamma$  subunits is opposed by ATP. Generally, the ratio of AMP to ATP ratio is an indicator of AMPK activity (31). A decrease in the AMP to ATP ratio leads to increased AMPK activity during exercise (40-42).

AMP kinases play a number of roles in glucose utilization in tissues. In mammals insulin promotes lipid, protein, and glycogen synthesis, whereas AMPK inhibits these biosynthetic pathways. The effect of insulin on protein synthesis is mediated in part by activation of mTOR pathway via phosphorylation of TSC2, whereas activation of AMPK causes phosphorylation of different sites on TSC2 and inhibits mTOR (43,44); (**Figures 1.3 and 1.4**). Signaling via insulin or IGF-1 affect AMP kinase activity. AKT, a protein kinase in the insulin and IGF-1 pathway, induces phosphorylation of AMPK at ser<sup>485</sup>/ser<sup>491</sup> which subsequently inhibits the phosphorylation of AMPK at thr<sup>172</sup> by LKB which suppresses AMPK activity. In addition, AMPK activation suppresses the signaling by insulin and IGF-1 pathway by phosphorylating insulin receptor substrate (IRS) at ser<sup>794</sup> and, as a result, inhibiting signaling of insulin and IGF-1 pathway to AKT mTOR in smooth muscle (45). Winder and Hardie (46) have suggested that AMPK activators may be useful in the treatment of Type II diabetes.

AMPK also stimulates glucose transport and glycolysis. In the heart, glucose uptake is mediated primarily by GLUT4. AMPK stimulates GLUT4 translocation to the sarcolemma (47) via an indirect mechanism. Phosphorylation of Rab GTPase-activating proteins regulate Rab10,

which modulates docking and fusion of GLUT4 vesicles with the plasma membrane. In addition, AMPK may inhibit endocytosis of GLUT4 in cardiomyocytes thus increasing sarcolemma GLUT4 content and glucose transport (48).

AMPK also affects glucose metabolism by indirectly increasing the activity of phosphofructokinase-1, the rate-limiting enzyme in glycolysis. Activated AMPK directly phosphorylates and stimulates phosphofructokinase-2 to synthesize fructose 2, 6-bisphosphate, which in turn allosterically activates phosphofructokinase-1. Thus increases in myocardial glucose transport and glycolysis are important components of the metabolic response to ischemia or hypoxia. Insulin and AMPK can also cooperate to regulate plasma glucose levels. In skeletal muscle, both insulin and AMPK activation stimulates glucose uptake via increased translocation of GLUT4 to the plasma membrane.

AMPK activity is affected by aging, probably resulting from its interactions with mTOR (49). With aging, there are reported increases in the AMP/ATP ratio (50-52), lower levels of AMPK in muscle (53,54) and a decrease in the responsiveness of AMPK signaling (55). Moreover, the baseline activity of AMPK was higher in older animals compared to younger animals (54). Such changes may contribute to a decrease in AMPK responsiveness to metabolic stress. These observations are also consistent with the “rate-of-living” theory of aging that hypothesizes that excessive consumption of energy enhances the aging process.

## **1.6: Changes in metabolic hormones associated with aging and insulin resistance**

Changes in pancreatic function, insulin receptor numbers and post-receptor changes in insulin-mediated signaling contribute to insulin resistance seen in aging. Strategies for compensating for these aging-related changes include dietary changes, exercise, oral

hypoglycemic agents and, with more severe forms, use of insulin. In addition, metabolic activity associated with thyroid function is also affected by aging which can result in lower plasma thyroxine (T4) and increased thyrotropin stimulating hormone (TSH). This is seen in approximately 5 to 10% of older women (56) and may be the result of autoimmune disease rather than a direct consequence of aging (57,58). There are also decreases in growth hormone release from the pituitary gland and, as a result, a decrease in IGF-I production by the liver.

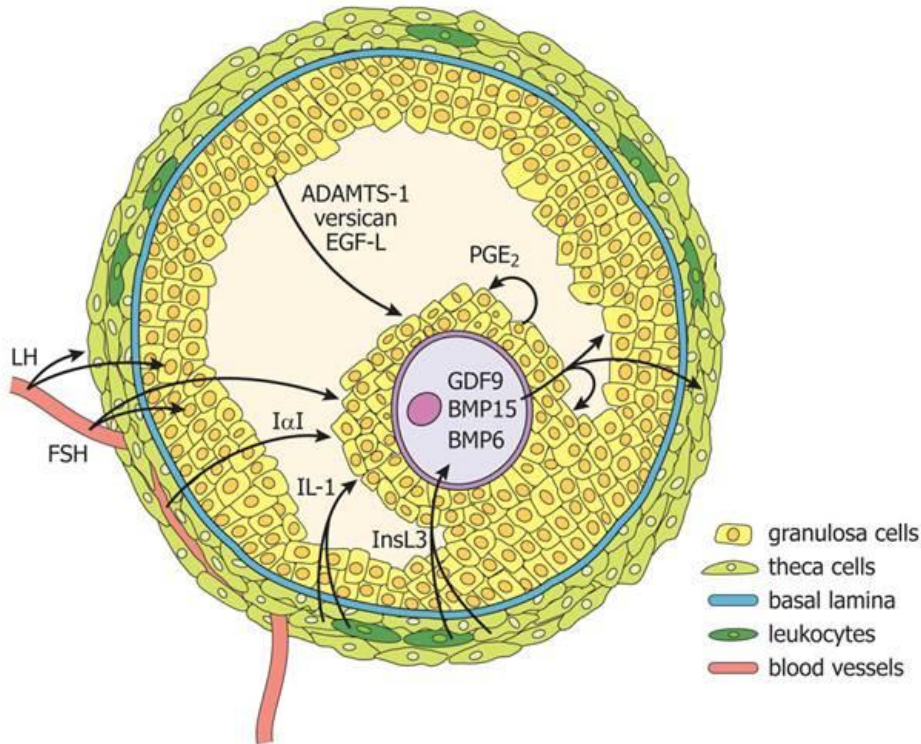
Reductions in growth hormone with aging have direct effects on ovarian function. It binds receptors on granulosa, thecal and luteal cells. Growth hormone and IGF-1 increase the sensitivity of ovaries to gonadotropin stimulation and enhance follicular development. Both IGF-1 and IGF-2 affect the maturation of the follicle and gamete as well (59). When growth hormone levels decrease, low serum levels of IGF-I prevent the dominant follicle from forming and, in women, can result in multiple ovulatory follicles. Increasing IGF-1 levels restores selection of a single dominant follicle (60).

### **1.7: Do changes in metabolic function accompany aging in mares?**

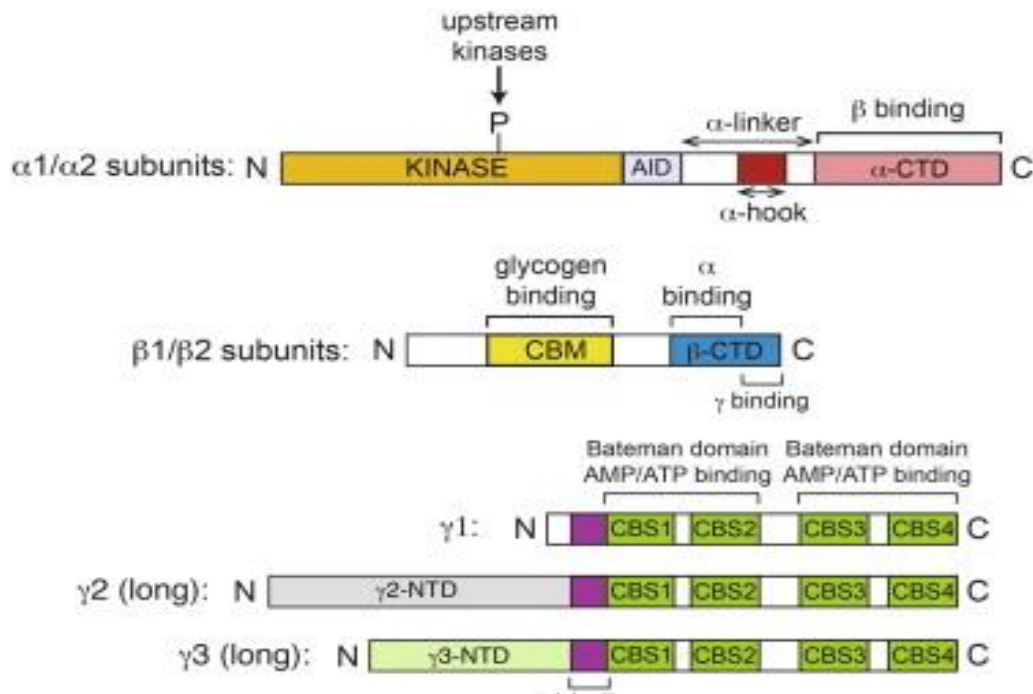
These various studies suggest that aging in mares is accompanied by metabolic changes as well as changes in ovarian function. The goal of this study was to evaluate the effects of aging on expression of genes responsible for hormone responsiveness in granulosa cells in the dominant follicle and metabolic activity of those cells in response to both aging and hormone treatment. This involved selection of genes involved in insulin-mediated effects on aging and analysis of gene expression in granulosa cells from mares of various ages using RT-PCR.

## **1. 8: Hypothesis**

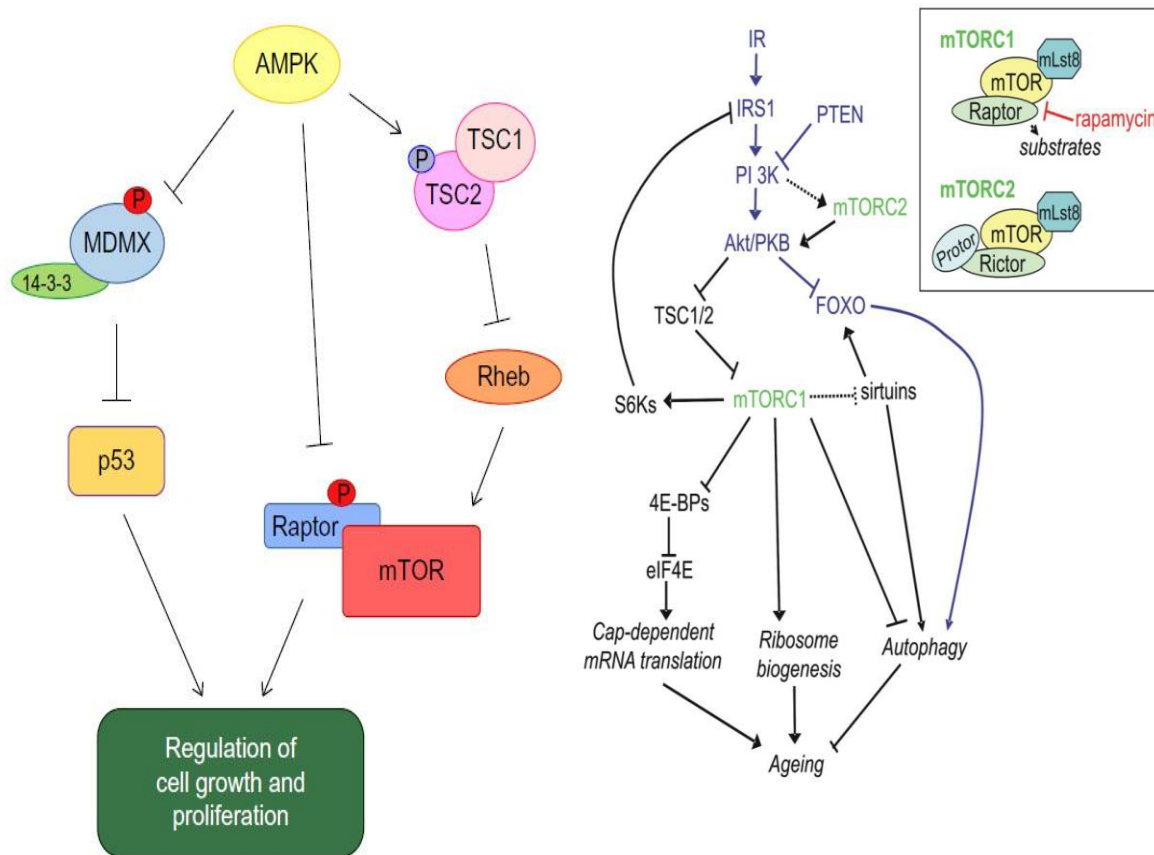
We hypothesize that granulosa cells in older animals express less insulin receptor, insulin-like growth factor-1 receptor and GLUT4 genes when compared to younger animals. These changes in gene expression affect the ability of older animals to respond to insulin and alter overall metabolic activity of these animals.



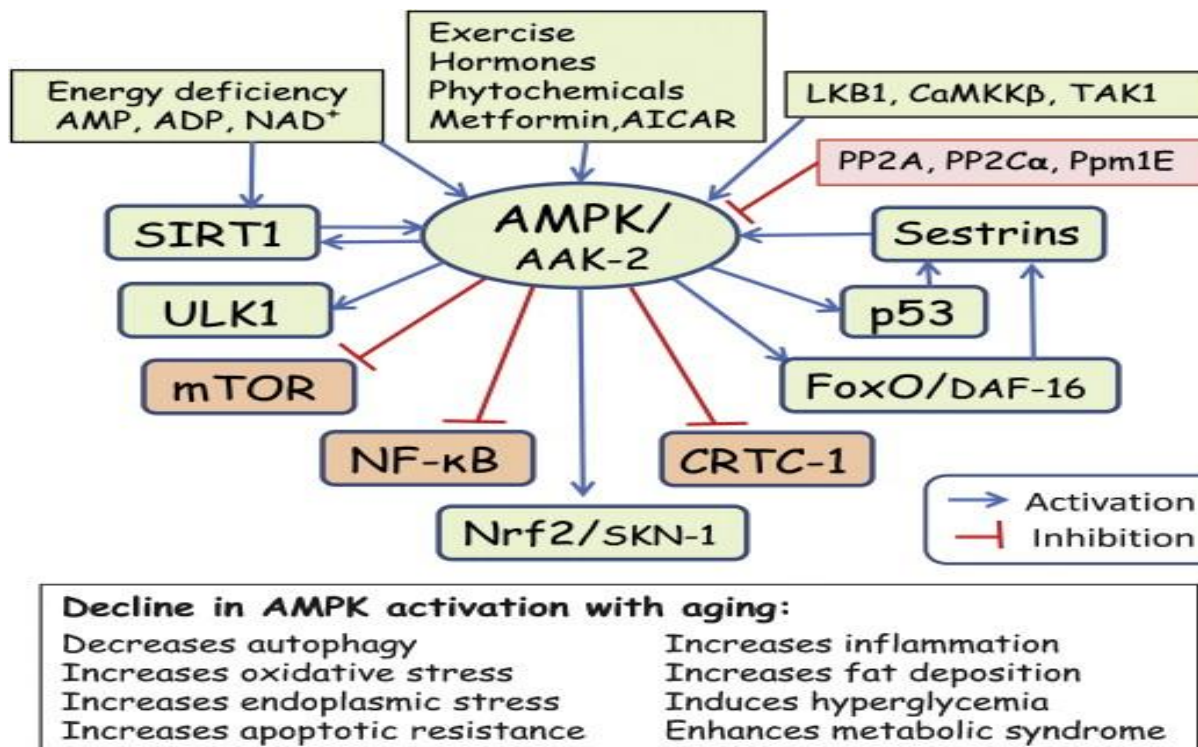
**Figure 1.1:** Signaling pathways and mediators of ovulation. Ovulatory surges of FSH and LH stimulate cells from multiple follicle compartments to transduce signals that collectively lead to ovulation. LH signaling is transduced by thecal and mural granulosa cells, whereas FSH signaling is transduced by mural granulosa and cumulus cells. Thecal cells secrete IL-1, a cytokine that stimulates cumulus cell expansion, and InsL3, a peptide that may trigger a reduction in oocyte cAMP levels. Mural granulosa cells secrete EGF-like ligands, growth factors that transduce the ovulatory response to the cumulus cells. In addition, they secrete versican and AdamTS-1, both of which become components of the cumulus cell matrix. PGE<sub>2</sub> generated by cumulus cells promotes cAMP production that enhances ovulatory signaling cascades. Oocyte morphogens including GDF-9, BMP-15, and BMP-6, and other signaling molecules generated both within and outside the follicle, modulate cumulus cell, mural granulosa cell, and theca cell responses to ovulatory signals. FSH, follicle stimulating hormone; LH, luteinizing hormone; IL-1, interleukin-1; InsL3, insulin-like peptide 3; cAMP, cyclic adenosine mono phosphate; EGF-L, EGF-like ligands; II, inter-trypsin inhibitor; PGE<sub>2</sub>, prostaglandin E2; GDF-9, growth and differentiation factor - 9; BMP-15, bone morphogenetic protein - 15; and BMP-6, bone morphogenetic protein - 6.



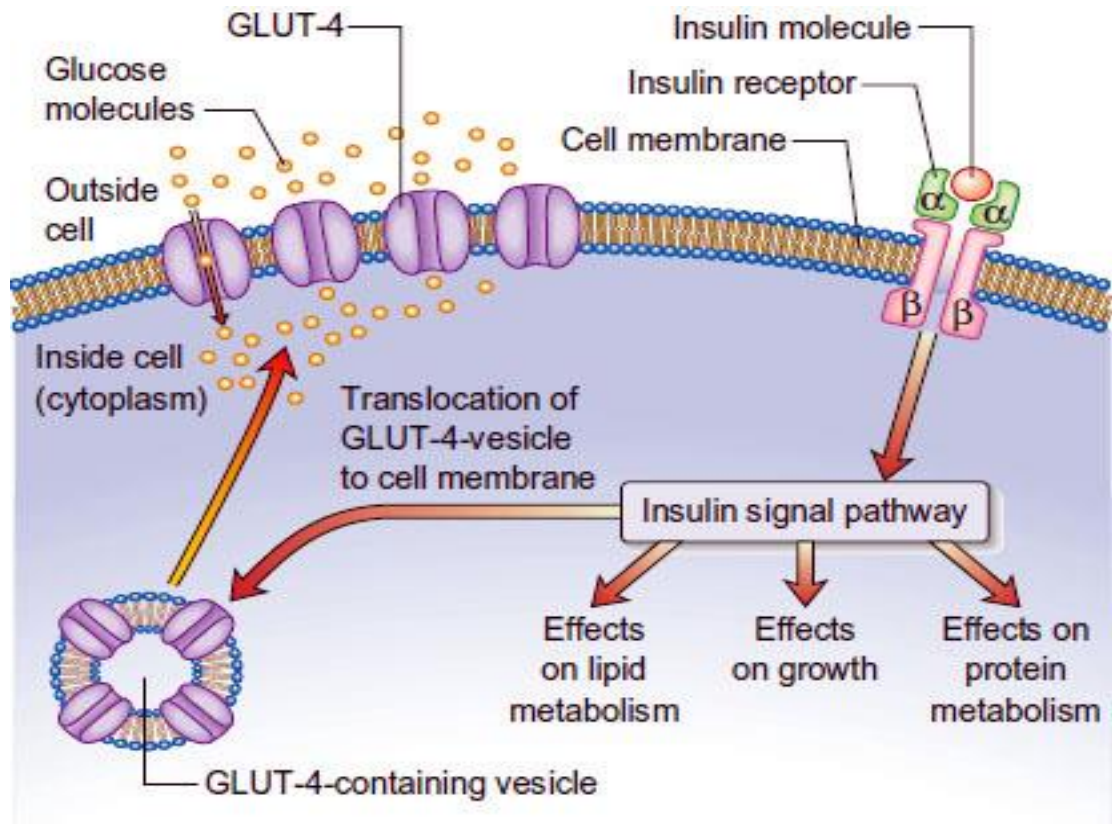
**Figure 1.2:** Domain structure of  $\alpha$ ,  $\beta$  and  $\gamma$  subunits of AMPK and their isoforms.  $\alpha$ -subunits; AID, autoinhibitory domain;  $\alpha$ -CTD, alpha C-terminal domain;  $\beta$ -subunits; CBM, carbohydrate-binding module;  $\beta$ -CTD, C-terminal domain;  $\gamma$  subunits; CBS1, CBS2, CBS3, and CBS4, cystathione  $\beta$ -synthase repeats;  $\gamma$ 2- NTD, N-terminal extension of “long” version of  $\gamma$ 2 subunit;  $\gamma$  3-NTD, N-terminal extension of “long” version of  $\gamma$  3 subunit.



**Figure 1.3.** AMPK modulates cell-cycle progression and cell growth through mTOR. mTOR is also involved in changes in insulin receptor function with aging.



**Figure 1.4:** Changes in AMPK function including inhibition of mTOR with aging. As shown here, AMPK activity can be stimulated by energy deficiency and several physiological and chemical agents including metformin which is used to treat insulin-resistance. The decline in the sensitivity of AMPK activation with aging disturbs metabolic balance and is associated with development of metabolic diseases. *Abbreviations:* AAK-2, AMP-activated kinase-2; AICAR, 5-aminoimidazole-4-carboxamide riboside; AMPK, AMP-activated protein kinase; CaMKK $\beta$ , Ca<sup>2+</sup>/calmodulin-dependent protein kinase kinase  $\beta$ ; CRTC-1, cyclic AMP-regulated transcriptional co-activator-1; DAF-16, abnormal dauer formation protein-16; FoxO, forkhead box protein O; LKB1, serine/threonine kinase 11; mTOR, mammalian target of rapamycin; NF- $\kappa$ B, nuclear factor- $\kappa$ B; Nrf2, nuclear factor-erythroid 2-related factor 2; PP, protein phosphatase; SIRT1, silent information regulator 1; SKN-1, skinhead-1; TAK1, transforming growth factor- $\beta$ -activated kinase 1; ULK1, UNC-51-like kinase 1.



**Figure 1.5.** Insulin and IGF-1 bind their receptors with high affinity and have effects on GLUT4 translocation to the plasma membrane. Increased signaling by these hormones promotes cell growth and energy metabolism. As shown for insulin receptor in this figure, both IGF-1 and insulin receptors are made up of two trans-membrane domain subunits with tyrosine-kinase activity. Any changes in these receptors and signaling is associated with variety diseases like cancer and diabetes mellitus.

## CHAPTER 2: MATERIALS AND METHODS

### 2.1: Introduction to RT-PCR

Mares are seasonal breeders and limited numbers of granulosa cells are available from a single animal. However, robust assays for granulosa cell function are now available that do not require large numbers of granulosa cells, e.g. reverse transcription-polymerase chain reaction (RT-PCR). Gene expression in response to various ligands as well as changes in metabolic activity may result from aging or development of metabolic disease. We used real-time reverse transcription polymerase chain reaction (RT-qPCR) to quantify gene expression in granulosa cells in response to FSH, LH, and EGF, insulin like growth factor-1 (IGF-1), insulin, and progesterone. We also examined effects of these ligands on expression of the glucose transporter type 4 (GLUT4), a glucose transporter that is up-regulated by insulin, and on the levels of adenosine monophosphate-activated protein kinase (AMPK) which reflects the metabolic activity of granulosa cells.

Polymerase chain reaction (PCR) following reverse transcription (RT) is a preferred technique to analyze mRNA expression using small numbers of cells. High sensitivity RT-PCR makes possible quantification of changes in gene expression and evaluation of expression of comparatively rare transcripts. There are a number of advantages to this method which combines comparatively simple methodology with rapid and reliable outcomes. RT-PCR, as used in our studies, indirectly detects mRNA levels following mRNA conversion to cDNA using a fluorescent dye, SYBR Green I that binds to the minor groove in double-stranded DNA (62).

The method used to quantify mRNA levels depends on the target sequence, the amount of mRNA in the sample, the level of accuracy desired and whether absolute or relative quantification

is needed (61). There are two primary methods for quantification of mRNA in RT-PCR studies. One method achieves relative quantification based expression of the target gene as compared to a reference gene. This technique is used to assess physiological changes in gene expression. There is also a second approach that provides absolute levels of a gene product based on either an internal or an external calibration curve (62,63). The use of a calibration curve helps validate the method and confirms whether there are reproducible levels of gene amplification for the mRNA standard and for the target cDNA (64-66).

In our studies, the relative levels of gene expression were determined by comparing the level of gene expression of particular gene of interest in a “treated” sample with the level of gene expression in an “untreated” sample. mRNA levels were examined using granulosa cells isolated from mature follicles following ovulation or aspiration. mRNA was reverse transcribed into cDNA using the script cDNA Super Mix 5X reaction that contains reverse transcriptase used to obtain cDNA. cDNA was placed in the LightCycle480 PCR system (Roche Applied Sciences) using a mixture of 1.0  $\mu$ l cDNA and 9.0  $\mu$ l of Master Mix in 384-well plates. Master Mix contains both the forward and reverse primers, SYBR Green I and water. RT-PCR proceeds through multiple cycle until the gene of interest is detected at a preset threshold. Abundant genes require fewer cycles for detection while less abundant genes require more cycles. A limit to the number of cycles can also be preset. In our studies, the PCR cycle conditions were as follows: 94 °C for 5 minutes, 40 cycles of 94 °C for 15 seconds, 60 °C for 30 seconds, and 72 °C for 15 seconds, and 72 °C for 3 minutes followed by a melt curve analysis to confirm amplification of single cDNA products. Raw Ct values, the number of cycles required for reaching threshold levels of detection, were normalized to internal control, and statistic differences were assessed at  $p < 0.05$ .

RT-PCR standards use reference or housekeeping genes such as glyceraldehyde-3-phosphate dehydrogenase (G3PDH or GAPDH), albumin, actins, tubulins, cyclophilin, 18S rRNA or 28S rRNA (67). These genes are present in nucleated cells, being necessary for cell survival. Although the constitutive synthesis of these genes is generally believed to remain stable under experimental treatments (67-69), some studies have shown that these levels of housekeeping genes may vary under experimental conditions (70-72). Nonetheless, for most applications, normalizing expression of target transcripts to an endogenous housekeeping gene transcript provides a reliable and accurate method for evaluating expression levels of the target gene.

To compensate for inter-PCR variations, normalization of target gene expression relative to an endogenous standard is the first step in assessing target gene expression. In addition, control levels of the standard are included in mathematical modeling of RT-PCR results to standardize each reaction. This allows the user to validate results with respect to the integrity of RNA, the efficiency of the reaction and/or variations in loading of the cDNA sample. In addition variation in the reproducibility of RT-PCR is almost impossible to eliminate due to the method used to isolate mRNA and the enzymes used in RT-PCR reactions. Nevertheless, the Light Cycler real-time PCR system using SYBR Green I fluorescence dye provides a reliable method to detect small amounts of mRNA and assess the level of mRNA expression.

## **2.2: Granulosa cell culture**

All procedures were done in accordance with the Colorado State University Institutional Animal Care and Use Committee. Mares were housed on dry lots with water and hay *ad libitum*. Reproductive tracts of the mares were examined using trans-rectal ultrasound. Dominant follicles were determined based on follicle growth and diameter, relaxed cervical tone and endometrial

edema. For pre-ovulatory follicle collections, follicular maturation was induced by administration of hCG and/or deslorelin (2500 IU and 1.5 mg, respectively, IV), and follicular contents were collected by transvaginal, ultrasound-guided follicular aspirations (73). Generally, equine granulosa cells were aspirated in mid-estrus from dominant follicles that were ~35 mm in diameter before induction of follicular maturation. The oocyte in this dominant follicle, together with granulosa cells from the follicle, was collected 30-34 h after induction of follicular maturation and prior to ovulation from young (4-9 years old), middle age (10-16 years old) and old age ( $\geq$  16 years old) estrous mares.

Granulosa cells were separated from tissue debris and red blood cells using Fico/Lite-LymphoH (Atlanta Biologicals, GA, Catalog # I40110) gradients and Manufacturer's instructions. Granulosa cells were maintained in plastic tissue flasks in Dulbecco's Modification of Eagle's Minimum Medium (DMEM; Mediatech, Inc., Manassas, VA), supplemented with 10% fetal bovine serum (FBS) (Gemini Bio Products, Woodland, CA), and 200 mM L-glutamine, 10,000 U/mL penicillin G, 10  $\mu$ g/mL streptomycin, and 25  $\mu$ g/mL fungizone streptomycin/mL (Gemini Bio-Products, Woodland, CA).

### **2.3: Cell treatments**

After cells were obtained from mares, cells were divided into two groups. One group of cells was serum starved (incubated without fetal bovine serum) and another group of cells was incubated with fetal bovine serum and 30mM glucose overnight. At the end of this incubation cell culture media was replaced by serum-free media before treating cells with either human epidermal growth factor (EGF; Sigma-Aldrich, E9644 SIGMA; 1  $\mu$ M), Recombinant human IGF-1 (Life Technologies, 10 nM), progesterone (PR, Sigma-Aldrich P0130-25G, 100 nM), insulin (Sigma-

Aldrich I-9278, 100 nM), or hCG (Fitzgerald, 100 nM) for 1 hour. We then harvested cells by soaking dishes in 5 mM EDTA for 5 minutes, removing the EDTA and washing cells using 1x PBS. Cells were washed using 15 ml centrifuge tubes and centrifugation at 300 x g for 3 min. This wash was repeated to eliminate any remaining supernatant. Isolated cells were then used immediately or were snap frozen in liquid nitrogen and stored at -80°C (74).

#### **2.4: Total RNA isolation**

RNA isolated from samples frozen at -80°C were treated with insulin, insulin like growth factor-1, EGF, human chorionic gonadotropin (hCG), epidermal growth factor (EGF), or progesterone (P4). RNA from these samples was then prepared using the RNeasy Mini kit (Qiagen Inc., Valencia, CA, Catalog #74104) according to the manufacturer's instructions followed by DNase digestion with RNase-Free DNase (Qiagen Inc., Valencia, CA, Catalog #79254) to remove any residual genomic DNA. The concentration of purified total RNA was determined by using a spectrophotometer at 260nm and 280nm (Nano Drop ND-1000, Nano Drop Technologies Inc., Rockland, DE). A ratio of readings at 260 nm and 280 nm (A260/A280) provides an estimate of the purity of RNA. Pure RNA has an A260/A280 ratio of 1.7 or greater. An A260/A230 ratio of 2.0 or greater was used to select samples for analysis (75), and samples were then stored at -80°C.

#### **2.5: Reverse transcription of mRNA**

Messenger RNA was reverse transcribed into cDNA using the qScript cDNA Super Mix (Quanta Biosciences, Inc., Gaithersburg, MD) 5X reaction. Reverse transcriptase was used to obtain cDNA (complementary DNA) from RNA that was extracted from our samples. We used 600ng of RNA per sample to obtain cDNA. Briefly, 5µl of RNA was combined with nuclease free

water and qScript Super Mix. RNA was reverse transcribed by incubating the samples at 25°C for 5 minutes, 42 °C for 30 minutes, and 28 °C for 5 min, cDNA was used immediately for real time PCR analysis.

## **2.6: Primer design, testing and sequencing**

Primers for ten equine genes, *AMPK $\alpha$ 1*, *AMPK $\alpha$ 2*, *AMPK $\beta$ 1*, *AMPK $\beta$ 2*, *AMPK $\gamma$ 1*, *AMPK $\gamma$ 2*, *AMPK $\gamma$ 3*, *IGF-1*, *GLUT4*, and *IR*, were designed using an online database at NCBI.nlm.nih.gov. Oligonucleotide primers were purchased from (Euro Fins, Huntsville, AL).

The primer sequences for the ten genes examined are shown in **Table 2.1**. Also the primer sequences for the three housekeeping genes (HK), GAPDH, ACTB, and SADH, are shown in **Table 2.2**. To ensure that only the target gene sequence was amplified, the primers were tested for efficiency by making a standard curve against pooled samples of equine granulosa cells that expressed the genes of interest, therefore serving as positive control. In addition PCR products were run on a 2% agarose gel and visualized using ethidium bromide. A Qiaquick Gel Extraction Kit (Qiagen, Valencia, CA, Catalog # 28704) was used to extract and purify DNA of 70bp to 10 kb from standard 2% agarose gel (**Figure 2.1**). These steps were performed to test for the accurate sequencing of specific primers and to verify that granulosa cells had the genes of interest to us. We also tested primers for their optimal temperature 56°C, 58°C, 60°C, and 62°C followed by use of gel separation to verify that the specific amplicon produced a single band of the correct size. The sample was washed with buffer PE to rinse away remaining gel and eluted in nuclease-free water. Samples were prepared to be sent for sequencing to the Colorado State University Proteomics Facility, Fort Collins, CO.

## **2. 7: Confirmation of primer specificity**

Specificity of RT-PCR products was documented with high resolution gel electrophoresis (**Figure 2.1**) and resulted in a single product with the desired length. Light Cycler melting curve analysis was also performed which resulted in a single product-specific melting temperature, No primer “dimers” were generated during 40 RT-PCR amplification cycles.

## **2. 8: Real-time PCR quantification of mRNA**

The relative expression of ten genes involved in insulin signaling and AMPK activity (*AMPK $\alpha$ 1*, *AMPK $\alpha$ 2*, *AMPK $\beta$ 1*, *AMPK $\beta$ 2*, *AMPK $\gamma$ 1*, *AMPK $\gamma$ 2*, *AMPK $\gamma$ 3*, *IGF-1*, *GLUT4*, and *IR*) and three genes serving as internal control (*GAPDH*, *ACTB*, and *SADH*) were examined using RT-PCR. Amplification efficiencies were determined using a 10-fold serial dilution series. Analysis was performed in 10  $\mu$ l reactions containing Sso Fast Eva Green Super mix (Bio-Rad, Hercules, CA), 0.5  $\mu$ M gene specific forward and reverse primer, and cDNA using a Roche Light Cycler® 480 Real-Time PCR System. Eva Green dye is a fluorescent nucleic acid dye that is not toxic with safe handling and is easily disposed. It is far brighter than SYBR green 1 for detecting amplification due to novel DNA binding mechanisms. It is stable during storage and under PCR conditions, in addition, electrophoretically separated PCR products can be visualized directly via a UV box without the need for another gel stain. To ensure highly specific amplification, Eva green is used because it has the ability to bind and detect any dsDNA generated during amplification. The sequence of temperatures used in gene amplification were as follows: 94°C for 5 minutes, 40 cycles of 94°C for 15 seconds, 60°C for 30 seconds, 72°C for 15 seconds, and 72 °C for 3 minutes. As discussed above, a melt curve analysis was performed to confirm amplification of single PCR products.

## **2. 9: Data analysis of RT-PCR**

The principle of RT-PCR quantification read-out is to determine the number of PCR cycles, i.e. the cycle threshold, Ct, as described by Schmittgen and Livak (76) and calculating the geometric mean of housekeeping genes expression values for use as a normalization factor. Ct is proportional to the logarithm of the initial amount of target in a sample. The relative concentration of one target with respect to another is reflected in the difference in cycle number,  $\Delta\text{Ct}$ , necessary to achieve the same level of fluorescence (76). We use the formula  $2^{-\Delta\text{CT}}$  to calculate  $\Delta\text{Ct}$  and to normalize our data. Prior to beginning these experiments, we confirmed our primer efficiency and specificity via PCR and sequencing (Go-Tag, Promega). We compared the level of gene expression of particular gene of interest in treated sample relative to the level of gene expression in untreated samples. Fold-change between treated and untreated samples was calculated as  $2^{-\Delta\Delta\text{Ct}}$ .

## **2.10: Statistical analysis**

SAS mixed procedure for repeated measures analysis was conducted for these data. Analysis was done using SAS Proc Mixed. A total of n=18 horses were included in this study (six young mares, four middle aged mares and eight older mares). A repeated measure's analysis was conducted for each gene (10 levels) and control +/- FBS and 30nM glucose separately. The response used log transformed expression to satisfy the assumption of normality. The "between subjects" factor was age (young, middle-aged and old mares) and the "within subjects" factor was treatment (12 levels). Tukey adjusted pairwise comparisons of treatments were considered when

Tukey adjusted pairwise comparisons were used. Dr. Hess also validated that the log transform of our data helped to satisfy model assumptions.

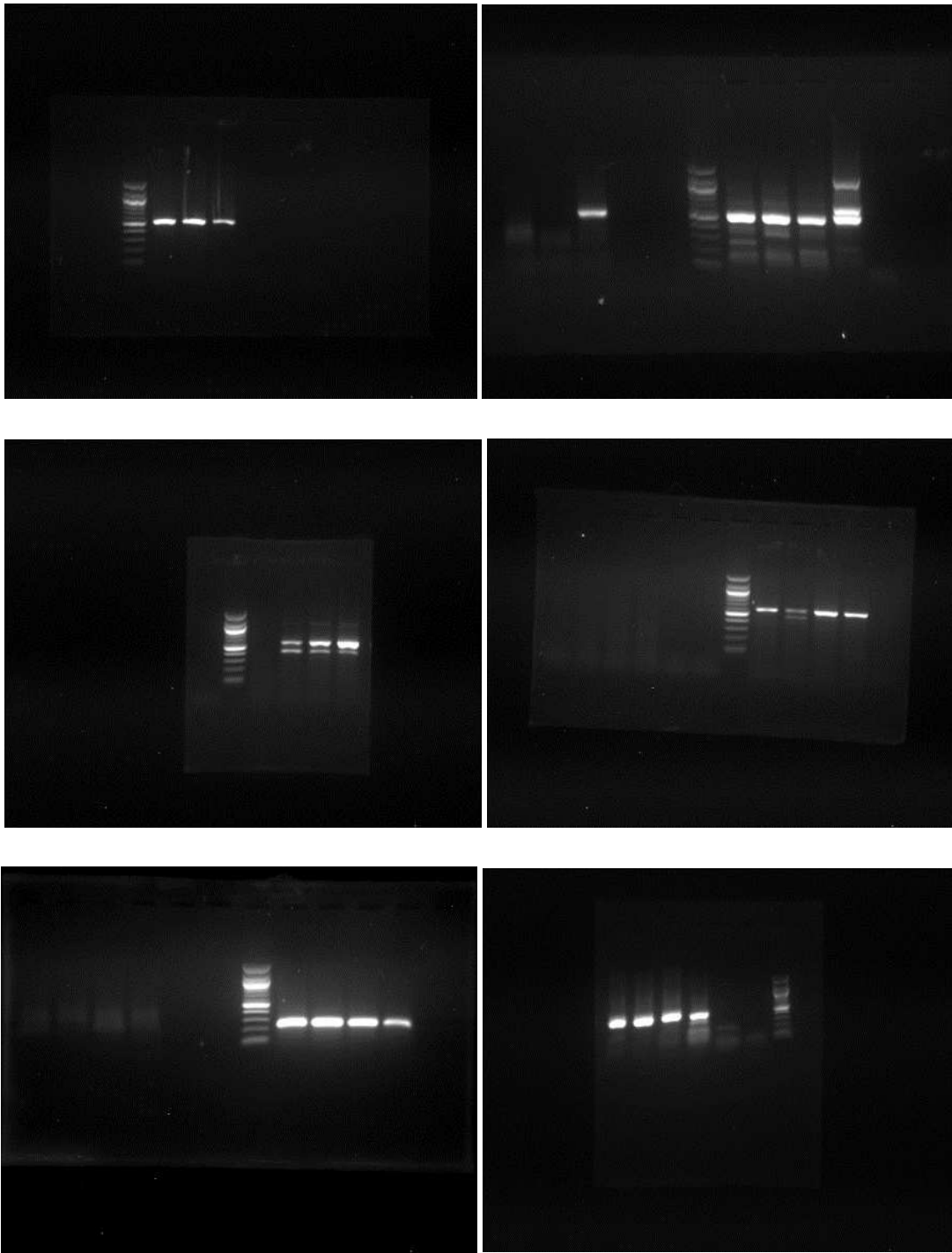
Dr. Hess also performed the following tests. **TEST 1** gave the ANOVA F-tests for each variable. **TEST 2** provided pairwise comparisons on main effects (Age Category or Age) for each variable or gene. The medium used for preincubation is indicated in her analyses as “m” for cells incubated in –FBS/-30nM glucose medium and “p” for cells incubated in +FBS/+30nM glucose. **TEST 3** gave slice comparisons or interaction comparisons. As a caution, Dr. Hess indicated that back transformed means might be substantially lower than the means in the original scale, particularly when the transformation is of  $\ln y$ . In addition, there were instances where adding a constant was required to calculate log values when there were zeros in the data. A summary of Dr. Hess’ statistical analysis is included in **Appendix I, II, and III.**

**Table 2.1:** Sequences of the ten genes of interest used in this project including *AMPK $\alpha$ 1*, *AMPK $\alpha$ 2*, *AMPK $\beta$ 1*, *AMPK $\beta$ 2*, *AMPK $\gamma$ 1*, *AMPK $\gamma$ 2*, *AMPK $\gamma$ 3*, *IGF-1*, *GLUT4*, and *IR*.

<b>Equine Gene</b>	<b>Sequence</b>	<b>Amplicon size bp</b>
<i>eIGF1-F</i>	<i>CTCTTCTACCTGGCCCTGTG</i>	225
<i>eIGF1-R</i>	<i>CTCCAGCCTCCTCAGATCAC</i>	
<i>eIR-F</i>	<i>CTACCTGGCCACCATTGACT</i>	226
<i>eIR-R</i>	<i>GCAGCCGTGTGACTTACAGA</i>	
<i>eGLUT4 F</i>	<i>GGTCATCAATGCCCCACAGA</i>	573
<i>eGLUT4 R</i>	<i>CGGCTGATGTAGAGGTAGCG</i>	
<i>eAMPK alpha1-F</i>	<i>GACAGCCGAGAAGCAGAAAC</i>	223
<i>eAMPK alpha1-R</i>	<i>AGGATGCCTGAAAAGCTTGA</i>	
<i>eAMPK alpha2-F</i>	<i>GACGGGTTGAAGAGATGGAA</i>	250
<i>eAMPK alpha2-R</i>	<i>CCTGCATACAATCTGCCTGA</i>	
<i>eAMPK beta 1-F</i>	<i>CGACCCTTCTGAGCCAATAG</i>	239
<i>eAMPK beta 1-R</i>	<i>GGATGACCTGGAGCAGATGT</i>	
<i>eAMPK beta2-F</i>	<i>ACTTTGTTGCCATCCTGGAC</i>	219
<i>eAMPK beta2-R</i>	<i>AGCTGGAAAGGTCTCGACAA</i>	
<i>eAMPK gamma1-F</i>	<i>GGGTGTCCTCAAGTGCTACC</i>	506
<i>eAMPK gamma1-R</i>	<i>GGTGAGGTGTGGGATCTCT</i>	
<i>eAMPK gamma2-F</i>	<i>GTAGCCAATGGTGTTTCGTGC</i>	501
<i>eAMPK gamma2-R</i>	<i>CACTACAGGCAAGGCTGACA</i>	
<i>eAMPK gamma3-F</i>	<i>GCTGGTCATCTTCGACACCA</i>	519
<i>eAMPK gamma3-R</i>	<i>AAGATGTCCAGTGCGGTCAG</i>	

**Table 2.2:** Sequences of the three housekeeping genes used in this project.

	<b>Equine Gene</b>	<b>Sequence</b>	<b>Amplicon size bp</b>
1.	<i>SDHA-F</i>	<i>GCAGAAGAAGCCATTTGAGG</i>	223
	<i>SDHA-R</i>	<i>CCTGTCGATTACGGGTCTGT</i>	
2.	<i>ACTB-F</i>	<i>CGACATCCGTAAGGACCTGT</i>	250
	<i>ACTB-R</i>	<i>CAGGGCTGTGATCTCCTTCT</i>	
3.	<i>GAPDH-F</i>	<i>AGAAGGAGAAAGGCCCTCAG</i>	239
	<i>GAPDH-R</i>	<i>GGAAACTGTGGAGGTCAGGA</i>	



**Figure 2.1:** Images of gel electrophoresis using 2% agarose gel and visualization of primers using ethidium bromide staining of the primers and base pair molecular markers. Each panel shows a single band of the correct size for the gene being examined.

## CHAPTER 3: RESULTS

### 3. 1: Introduction

The data presented here were obtained from mares within the three age groups described in Materials and Methods. For each animal, a complete data set was obtained which included  $\Delta Ct$  values for cells pre-incubated in either -FBS/-30nM glucose or +FBS/+30nM glucose medium and subsequently treated with insulin, IGF-1, EGF, P4 or hCG. The complete results for each pre-incubation condition and treatment are summarized in **Tables 3.4 - 3.13** at the end of this chapter.

### 3. 2: Comparison of the effects of medium containing -FBS/-30nM glucose and medium containing +FBS/+30nM glucose on $\Delta Ct$ values for *IR*, *IGF-1R* and *GLUT4* expression.

With the exception of significantly lower *IR* expression in granulosa cells from older animals pre-incubated with +FBS/+30nM glucose compared to cells pre-incubated in -FBS/-30nM glucose ( $p < 0.02$ ),  $\Delta Ct$  values for *IGF-1R*, *IR* or *GLUT4* expression did not differ significantly for otherwise untreated granulosa cells from young, middle or older animals pre-incubated in medium containing either -FBS/-glucose or +FBS/+30nM glucose (**Figure 3.1**).

**Figure 3.2** summarizes the values for *IR* gene expression in control granulosa cells and in hormone-treated cells preincubated with medium containing -FBS/-30nM glucose or +FBS/+30nM glucose. There was no significant effect of hormone treatment in cells pre-incubated in medium containing +FBS/+30nM glucose. There was, however, a significant increase in *IR*

expression following treatment with insulin in older animals compared to treatments with hCG, P4, EGF or IGF-1 (**Figure 3.3**) when FBS and glucose were absent from pre-incubation medium. The insert in **Figure 3.3** shows values for mean  $\ln y$  where  $y = 2^{-\Delta\Delta Ct}$  which were used for statistical analysis of these results.

**Figure 3.4** summarizes the values for *IGF-1R* gene expression in control granulosa cells and with hormone treatment for cells preincubated with medium containing -FBS/-30nM glucose or +FBS/+30nM glucose. As shown in **Figure 3.5**, there was a significant increase ( $p < 0.03$ ) in expression of *IGF-1R* in older animals (>16 years) relative to young aged mares (4-9 years) and middle-aged mares (10-16 years) in medium containing +FBS/+30nM glucose. The insert in **Figure 3.5** shows the values for the mean  $\ln y$  where  $y = 2^{-\Delta\Delta Ct}$  for middle and older animals. There were also significantly more *IGF-1R* expressed in EGF-treated cells compared to IGF-1-treated cells ( $p < 0.03$ ) in granulosa cells from older animals incubated in medium containing +FBS/+30nM glucose (**Table 3**). There were no significant differences in effects of hormone treatments on IGF-1R expression for any age groups (**Figure 3.4**).

### **3. 3: Effect of *IR* and *IGF-1R* expression on the predicted numbers of insulin receptors and IGF-1 receptors assembled in individual animals.**

To examine the relationship between *IR* and *IGF-1R* expression in *individual* animals,  $\Delta Ct$  values were expressed as a ratio of the  $\Delta Ct$  for *IR* to the  $\Delta Ct$  for *IGF-1R* for each animal and then pooled by age (**Table 3.1**). This was done for granulosa cells incubated without (-) or with (+) FBS/30nM glucose and for untreated cells and cells treated with 100 nM insulin or 100 nM IGF-1 100 nM hCG or 1  $\mu$ M EGF.

Assuming that assembly of IR homodimers, IGF-1R homodimers and IR·IGF-1R hybrid receptors is dependent on available IR and IGF-1R monomers, a ratio of  $\Delta\text{Ct}$  for *IR* relative to  $\Delta\text{Ct}$  for *IGF-1R* of 1.0 could be expected to produce assembly of equal numbers of IR<sub>2</sub> homodimers, IR·IGF-1R hybrid receptors and IGF-1R<sub>2</sub> homodimer in the endoplasmic reticulum (77). When ratios of  $\Delta\text{Ct}$  for *IR* relative to  $\Delta\text{Ct}$  for *IGF-1R* are greater than 1.0, synthesis of IR monomers is expected to be lower than synthesis of IGF-1R monomers from these genes in the endoplasmic reticulum. Thus there will be increased formation of IGF-1 receptor homodimers and IR·IGF-1R hybrid receptors compared to insulin receptor homodimer formation such that IGF-1R<sub>2</sub> = IR·IGF-1R hybrid receptors > IR<sub>2</sub>. Similarly, when ratios of  $\Delta\text{Ct}$  for *IR* relative to *IGF-1R* are less than 1.0, synthesis of the IR monomer expression is increased relative to IGF-1R monomer and there is a predominance of IR<sub>2</sub> homodimers assembled in the endoplasmic reticulum. Individual young animals thus differ significantly from middle-aged and older animals with respect to relative expression of *IR* and *IGF1R* genes. In untreated granulosa cells from young animals, values for the ratio of  $\Delta\text{Ct}$  of *IR* to  $\Delta\text{Ct}$  of *IGF-1R* ranged from 1.26-1.54 suggesting that there may be increased overall synthesis of IGF-1R monomers in the endoplasmic reticulum and, as a result, an increase in assembly of IGF-1R<sub>2</sub> homodimers and hybrid receptors. By contrast, in control granulosa cell populations of older animals (+FBS/+30nM glucose), the ratio is about  $0.89 \pm 0.19$ . We would expect that IR<sub>2</sub> homodimers would be the predominant receptor assembled and expressed in the plasma membrane.

### **3. 4: Effects of the *IR: IGF-1R* on *GLUT4* expression.**

The question raised by the above results is whether granulosa cell function differs as a result of these age-dependent shifts in the ratio of  $\Delta\text{Ct}$  for *IR* relative to  $\Delta\text{Ct}$  for *IGF-1R*. Results

for *GLUT4* expression in pre-incubation medium containing either -FBS/-30 nM glucose or +FBS/+30 nM glucose are shown in **Figure 3.6**. *GLUT4* expression differed significantly between young and older animals ( $p < 0.05$ ) in +FBS/+30 nM glucose -containing medium (**Figure 3.7**). Older animals demonstrated higher levels of *GLUT4* expression for each of the treatments shown including insulin treatment. Whether this is due to higher levels of *IR* expression or other properties of granulosa cells is not known. Middle-aged animals, predicted to express higher overall levels of *IR* related to *IGF-1R*, did not show a similar response. It is also important to note that, in medium without FBS or glucose, there was no significant difference in the expression of *IR* or *IGF-1R* or the ratio of *IR:IGF-1R* for any age group.

### **3. 5: Effects of aging or hormone treatment on AMPK subunit expression**

We also examined expression of various subunits of AMP-activated protein kinase including  $\alpha 1$  and  $\alpha 2$ ,  $\beta 1$  and  $\beta 2$  and  $\gamma 1$ ,  $\gamma 2$  and  $\gamma 3$  subunit isoforms. AMPK is a heterotrimeric kinase formed from  $\alpha$ ,  $\beta$  and  $\gamma$  subunits where the  $\alpha$  subunit is the catalytic subunit and the  $\beta$  and  $\gamma$  subunits serve regulatory functions (78). AMPK functions as a metabolic regulator that can switch usage of ATP during metabolic stress away from ATP-dependent anabolic pathways (79). There were age-dependent effects on expression of *AMPK  $\beta 1$*  (**Figure 3.8**). Young and older animals differed significantly in values for  $\Delta Ct$  *AMPK  $\beta 1$*  in the absence of FBS/30nM glucose ( $p < 0.006$ ) with higher expression of *AMPK $\beta 1$*  in older animals. Insulin treatment also affected *AMPK $\beta 1$*  expression following pre-incubation in either -FBS/-30nM glucose compared to +FBS/+30nM glucose ( $p < 0.03$ ) with higher expression in medium containing -FBS/-30nM glucose. These data are indicated by a bar connecting the two  $\Delta Ct$  values.

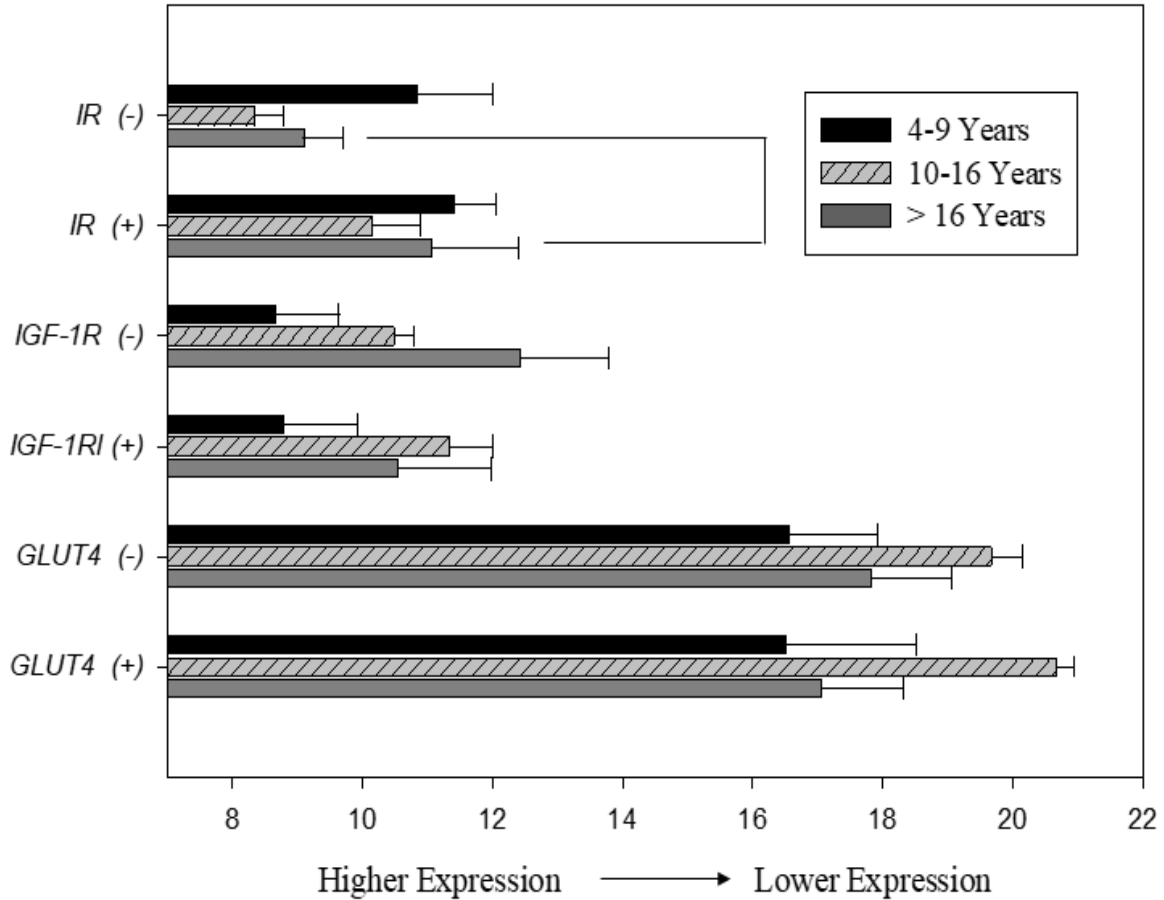
There were also significant age-related differences in expression of other *AMPK* subunits which are summarized in (**Table 3.4**). Noteworthy were differences in responses to insulin treatment in young or middle-aged animals and older animals for *AMPK β1* and *AMPK γ3* subunits where insulin treatment consistently increased expression in cells from older animals when cells were-pre-incubated in -FBS/-30nM glucose but not +FBS/+30nM glucose medium.

For middle aged and older animals but not young animals, differences in  $\Delta C_t$  values for expression of untreated cells or cells treated with insulin, hCG, EGF, IGF-1 or P4 were often significant within a given age group (**Table 3.5**). In middle-aged animals, there was significant differences in expression of a specific *AMPK* subunit when cells were pre-incubated in +FBS/+glucose medium. As examples, there were differences in the expression of *AMPK α1* gene between insulin-treated and EGF-treated cells ( $p < 0.04$ ), and insulin treatment and progesterone treatment ( $p < 0.03$ ) with higher expression of *AMPK α1* in response to insulin in both examples. Also, in middle-aged mares, there was greater expression of *AMPK α2* in cells pre-incubated in +FBS/+30 nM glucose medium in insulin-treated cells when compared to hCG treatments ( $p < 0.02$ ), EGF treatment ( $p < 0.01$ ), and progesterone treatment ( $p < 0.05$ ). In middle-aged animals, there were significant effects on *AMPK β1* expression following preincubation with +FBS/+30 nM glucose medium between control and EGF treatments ( $p < 0.004$ ), and between insulin and EGF treatments ( $p < 0.05$ ) insulin and progesterone treatments ( $p < 0.02$ ), and hCG and EGF treatments ( $p < 0.02$ ).

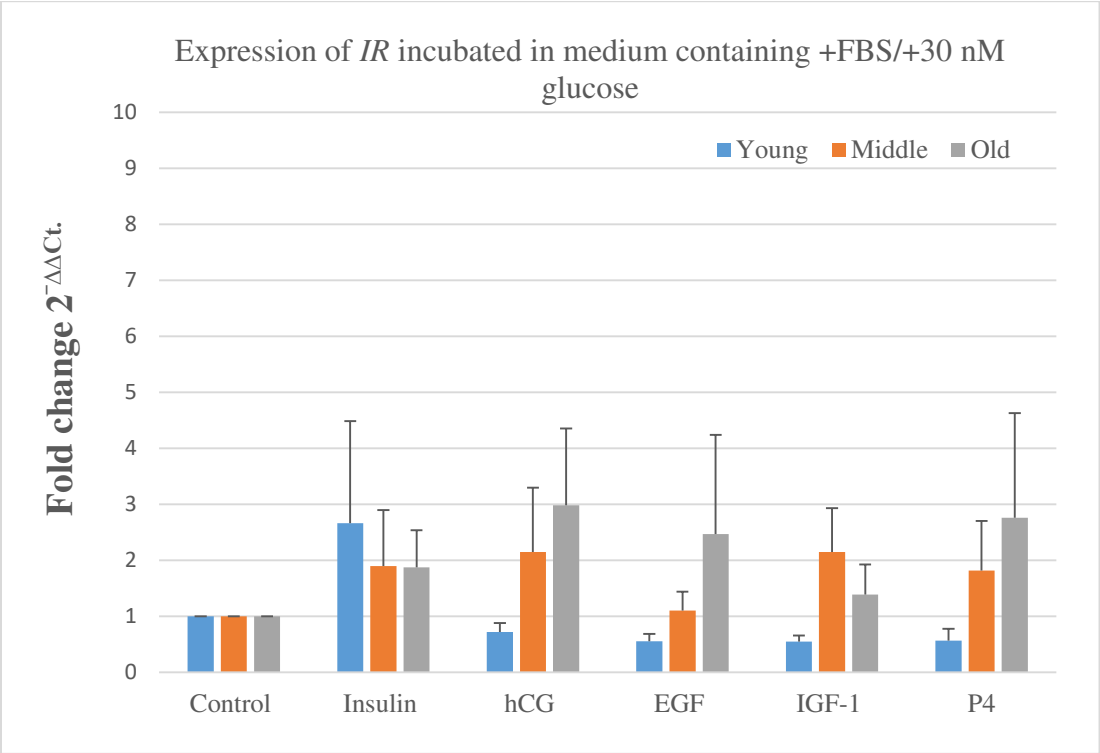
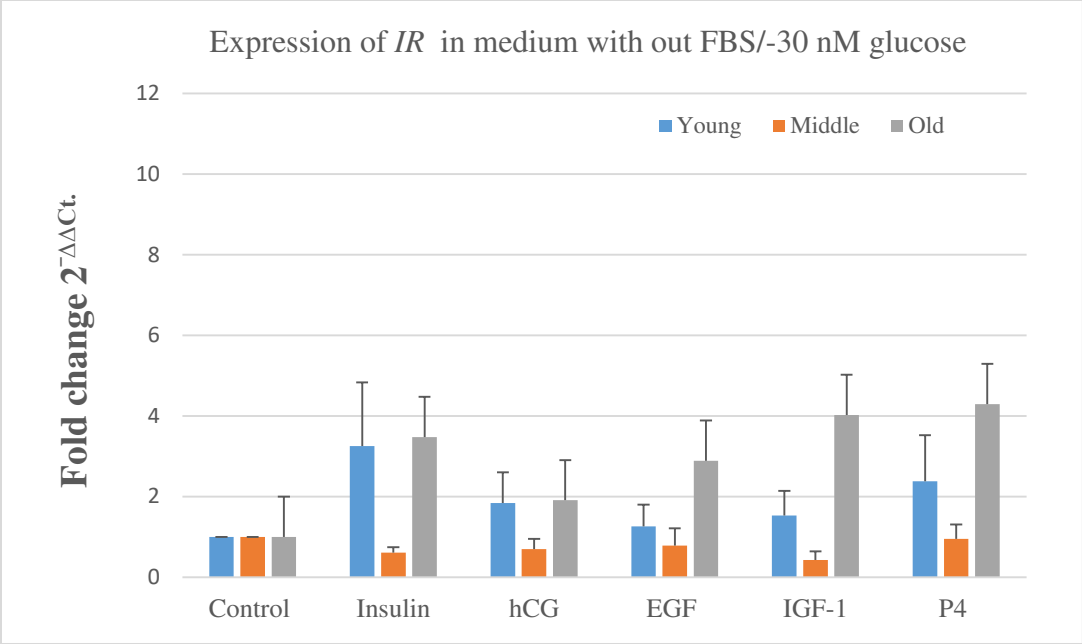
For expression of *AMPK β2* the only effect of treatment occurred in middle aged mares when cells were preincubated in +FBS/+30nM glucose medium between insulin and progesterone treatments ( $p < 0.02$ ). For *AMPK γ1*, there was no significant differences in expression for cells preincubated with or without FBS and 30nM glucose or for treated cells. In expression of *AMPK γ2*

in middle age there was a significant effect following pre-incubation in +FBS/+30 nM glucose medium when comparing treatment with IGF-1 and progesterone (P4) ( $p < 0.04$ ).

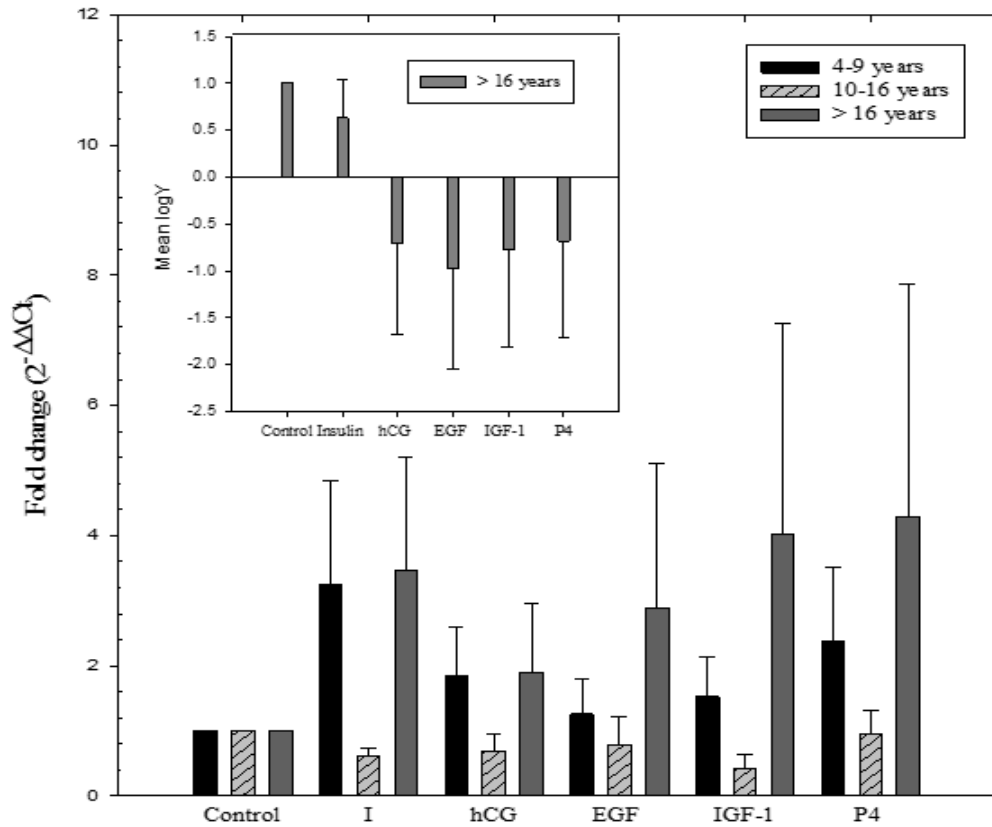
In older animals, AMPK $\gamma$ 2 gene expression was significantly different between control samples obtained from medium containing +FBS/+30 nM glucose and EGF-treated samples ( $p < 0.04$ ). AMPK $\gamma$ 3 expression was significantly affected in old aged animals pre-incubated in either in -FBS/-30 nM glucose or + FBS/+30 nM glucose media in old age. In absence of glucose and FBS there was a significant effect between insulin and EGF treatments ( $p < 0.001$ ), and between insulin and IGF-1 treatments ( $p < 0.03$ ) as well as between hCG and EGF treatments ( $p < 0.04$ ). In presence of FBS and 30nM glucose, there were significant effects between hCG and control ( $p < 0.02$ ), hCG and EGF treatments ( $p < 0.04$ ), and between hCG and IGF-1 treatments ( $p < 0.02$ ).



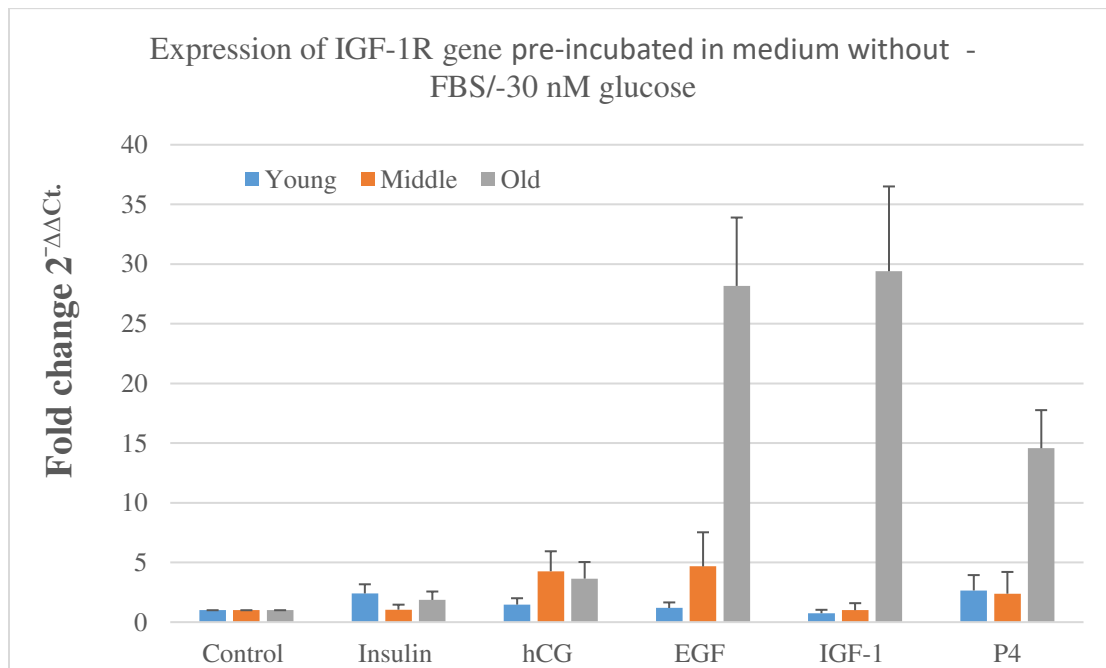
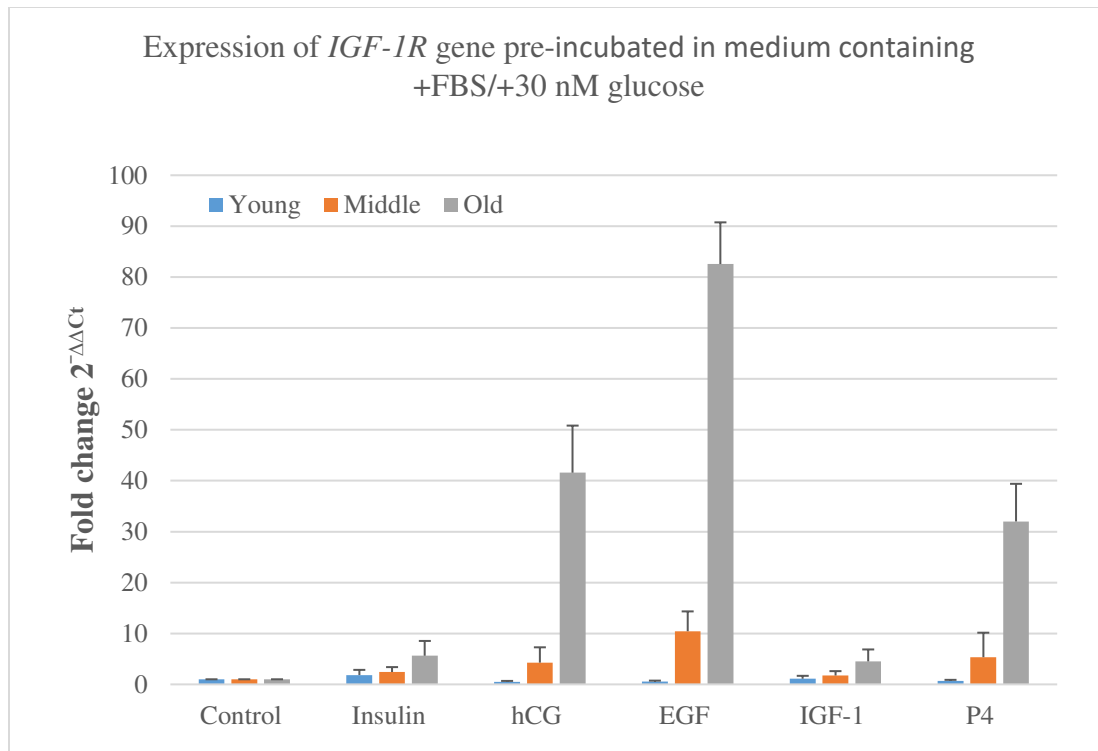
**Figure 3.1:**  $\Delta C_t$  values for *IR*, *IGF-1R* and *GLUT 4* expression in granulosa cells incubated with (+) or without (-) FBS and 30 nM glucose. As shown on the x-axis, lower values for  $\Delta C_t$  reflect higher expression of a particular gene. There was no significant difference between *IR*, *IGF-1R* or *GLUT4* expression between young, middle or old mares with the exception of *IR* expression in older animals where older animals express less *IR* following pre-incubation in medium containing +FBS/+30 nM glucose ( $p < 0.02$ ). This is indicated as a line between values for *IR* expression.



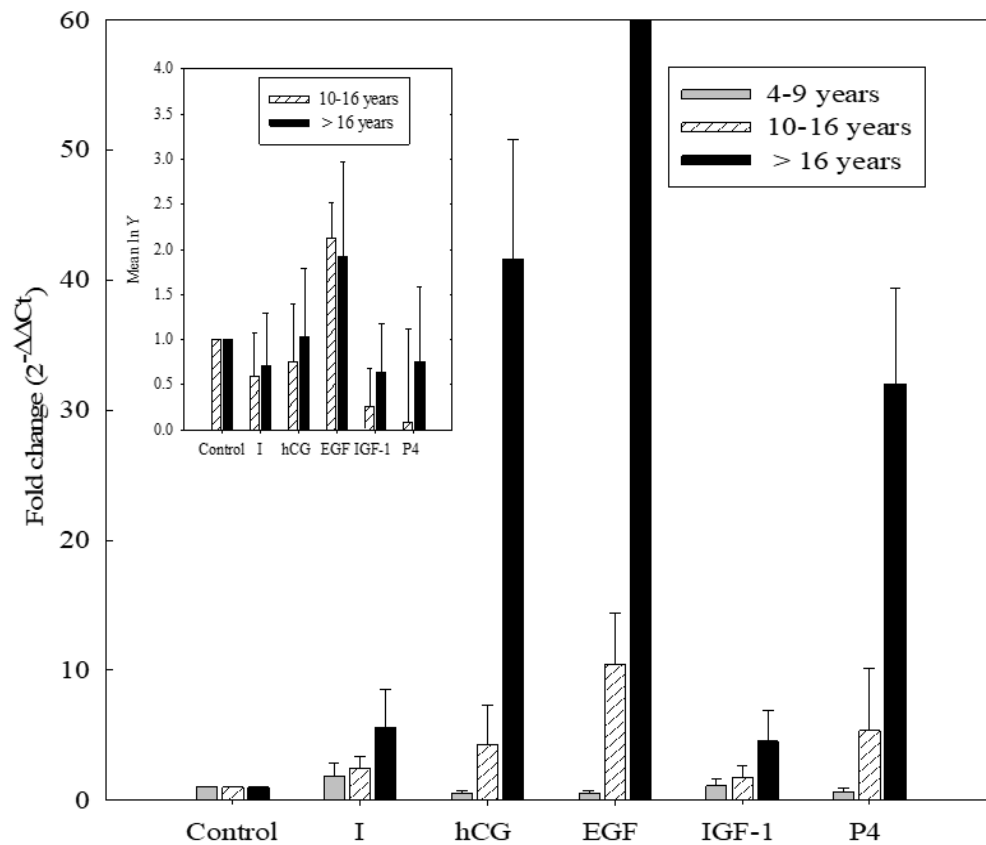
**Figure 3.2:** Fold change in *IR* expression in granulosa cells from young, middle, and old mares following pre-incubation in either +FBS/+30 nM glucose media or media without FBS/30 nM glucose .



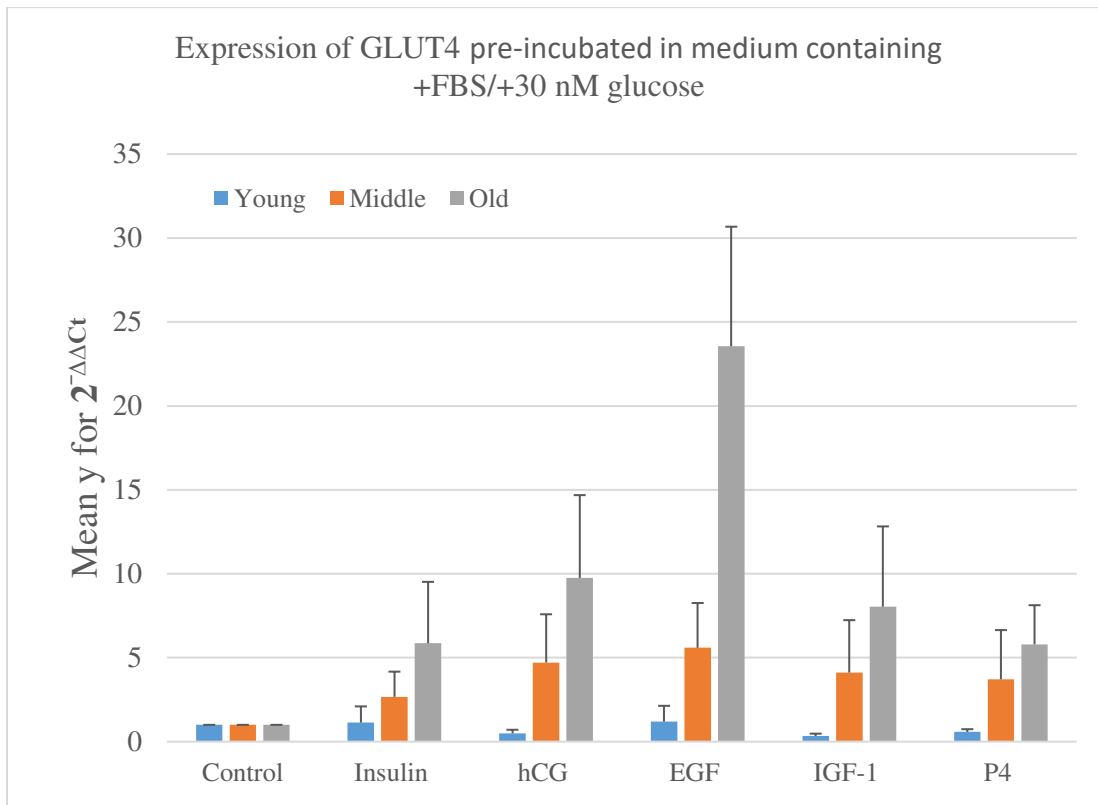
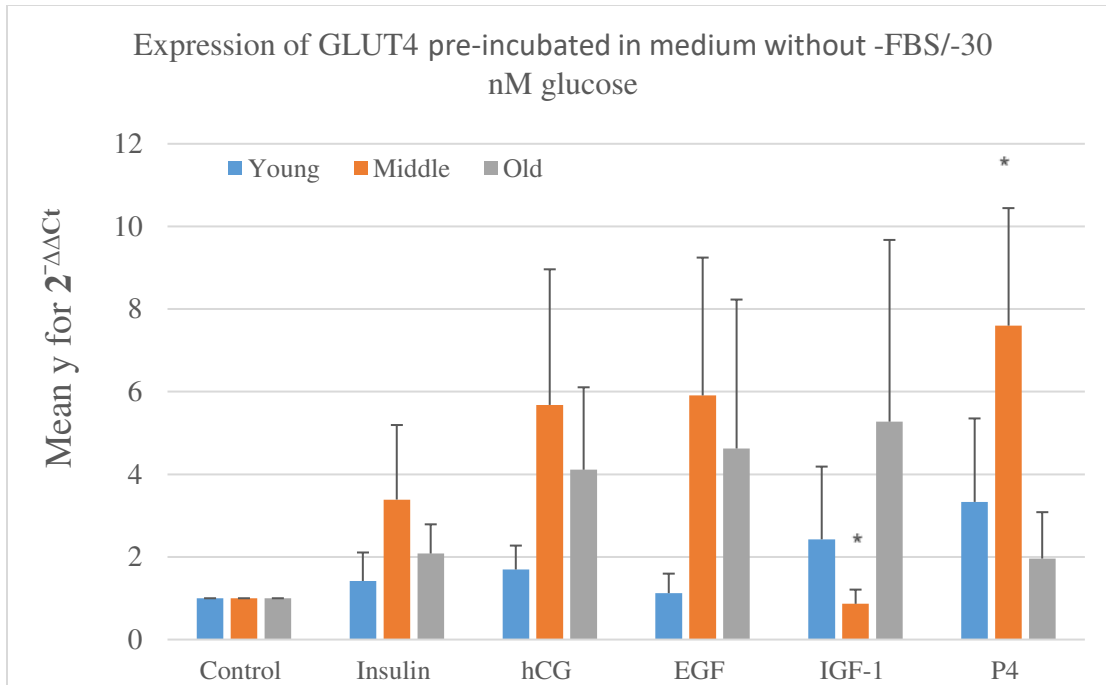
**Figure 3.3:** Fold change ( $2^{-\Delta\Delta C_t}$ ) in expression of *IR* relative to control values (1.0) with insulin, hCG, EGF, IGF-1 and P4 treatments for granulosa cells incubated in medium containing -FBS/-30nM glucose from young, middle and old animals. The insert shows mean  $\ln 2^{-\Delta\Delta C_t}$  which, for statistical purposes, was calculated for each animal. Using  $\ln 2^{-\Delta\Delta C_t}$ , old animals had statistically higher expression of *IR* with insulin treatment.



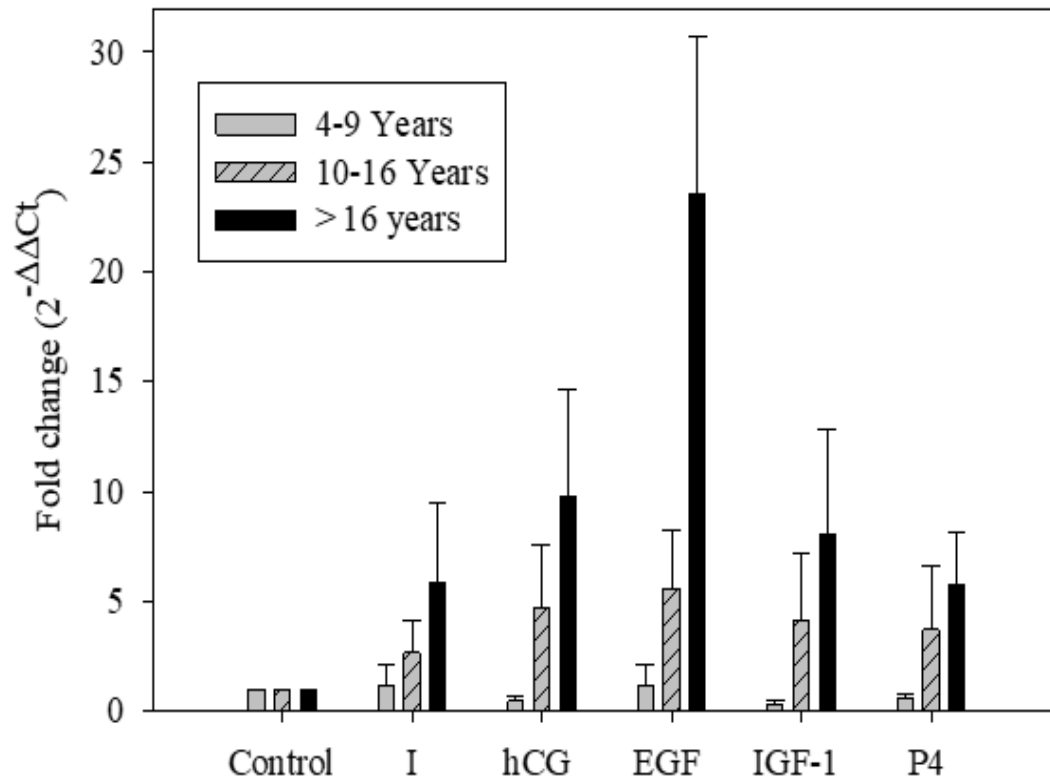
**Figure 3.4:** Fold change in *IGF-1R* expression in granulosa cells from young, middle, and old mares pre-incubated in media containing either +FBS/+30 nM glucose or -FBS/-30 nM glucose. Older animals had significantly higher expression of *IGF-1R*. Also, there was significant higher expression of EGF in middle and older animals compared to young animals.



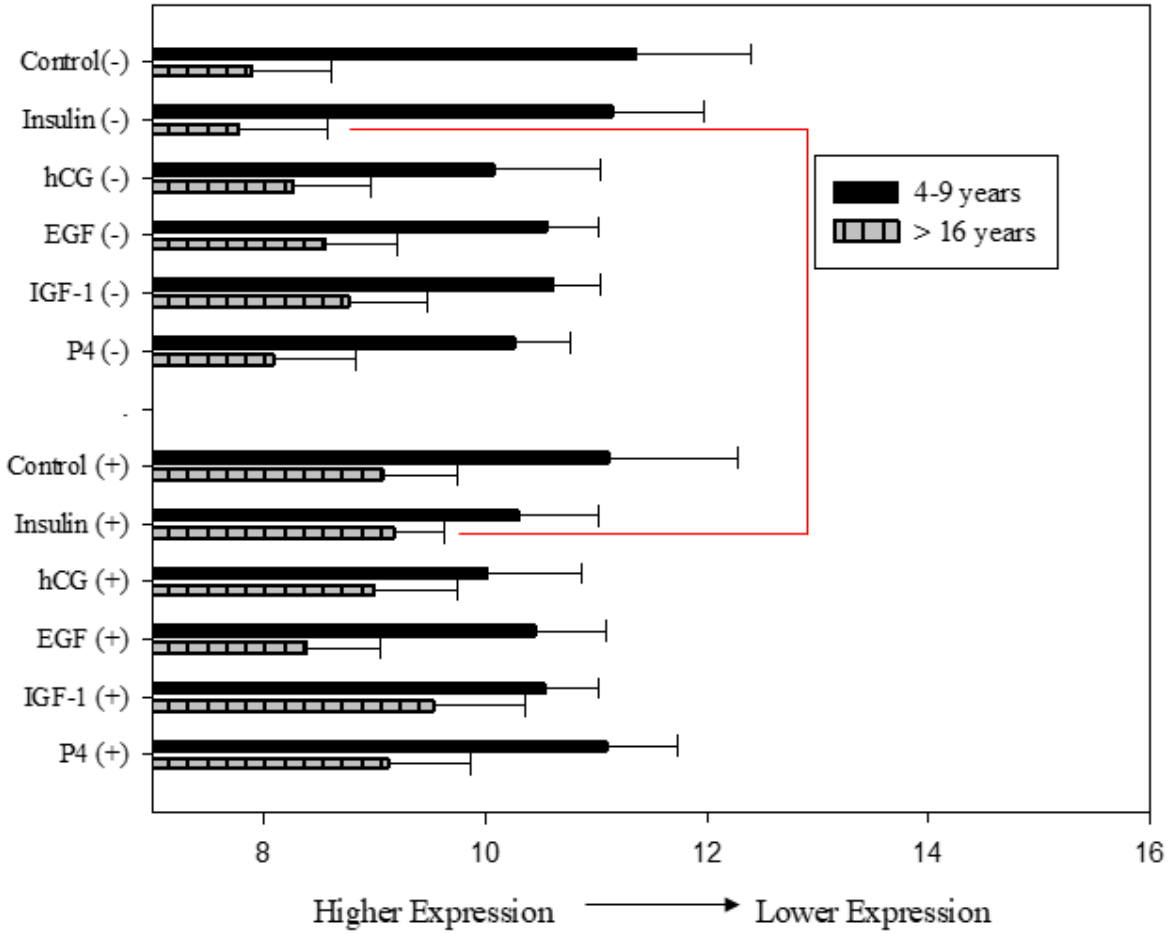
**Figure 3.5:** Fold change ( $2^{-\Delta\Delta C_t}$ ) in expression of *IGF-1R* relative to control values (1.0) with insulin, hCG, EGF, IGF-1 and P4 treatments for granulosa cells from young, middle and old animals incubated in medium containing FBS and 30nM glucose. The insert shows the mean  $\ln 2^{-\Delta\Delta C_t}$  values for granulosa cells from individual middle aged and old aged animals with EGF treatments which were used for statistical evaluation of these data.



**Figure 3.6:** Fold change in *GLUT4* expression in granulosal cells from young, middle, and old mares pre-incubated in media containing either +FBS/+30nM glucose (lower panel) or -FBS/-30nM glucose (upper panel).



**Figure 3.7:** *GLUT4* expression in granulosa cells from young and older mares incubated with +FBS/glucose medium. After overnight incubation, cells were treated with insulin, hCG, EGF, IGF-1 or progesterone (P4). EGF and IGF-1 significantly increased *GLUT4* expression in older animals compared to young animals ( $p < 0.05$ ).



**Figure 3. 8:** Expression of *AMPK β1* showing higher expression of *AMPK β1* in older mares ( $p < 0.006$ ). The red bar connecting  $\Delta Ct$  values for insulin treatment (-FBS/-30nM glucose) and insulin treatment (+FBS/+30nM glucose) indicates a significant difference in the effect of medium on *AMPK β1* with higher expression in cells pre-incubated in -FBS/-30nM glucose ( $p < 0.03$ ).

**Table 3.1:** The ratio  $\Delta\text{Ct (IR)}$  to  $\Delta\text{Ct (IGF-1R)}$  for *individual* animals and predicted effects of these ratios on expression of insulin receptor homodimers and IGF-1R homodimers. Note that, as  $\Delta\text{Ct}$  values *increase*, the amount of mRNA expressed *decreases*. Thus, ratios of  $\Delta\text{Ct (IR)}$  to  $\Delta\text{Ct (IGF-1R)} > 1$ , are predicted that IGF-1R monomers should be the predominant species expressed in granulosa cells and that these cells will contain increased numbers of IGF-1R and hybrid receptors formed from insulin and IGF-1R monomers. When ratios are  $< 1$ , insulin receptors will be the predominant receptors found on granulosa cells.

	Young Mares		Middle-aged Mares		Older Mares	
Treatment	$\frac{\Delta\text{Ct (IR)}}{\Delta\text{Ct (IGF-1R)}}$	Interpretation	$\frac{\Delta\text{Ct (IR)}}{\Delta\text{Ct (IGF-1R)}}$	Interpretation	$\frac{\Delta\text{Ct (IR)}}{\Delta\text{Ct (IGF-1R)}}$	Interpretation
Control (-)	$1.40 \pm 0.28$ (6)	↑ IGF-1R	$0.80 \pm 0.05$ (4)	↑ IR	$0.99 \pm 0.19$ (8)	IR ≈ IGF-1R
Control (+)	$1.53 \pm 0.35$ (6)	↑ IGF-1R	$0.88 \pm 0.08$ (4)	↑ IR	$0.89 \pm 0.19$ (8)	↑ IR
Insulin (-)	$1.54 \pm 0.21$ (6)	↑ IGF-1R	$0.86 \pm 0.09$ (4)	↑ IR	$1.22 \pm 0.42$ (8)	↑ IGF-1R
Insulin (+)	$1.30 \pm 0.11$ (6)	↑ IGF-1R	$0.96 \pm 0.09$ (4)	IR ≈ IGF-1R	$1.29 \pm 0.51$ (8)	↑ IGF-1R
hCG (-)	$1.51 \pm 0.50$ (6)	↑ IGF-1R	$1.02 \pm 0.15$ (4)	IR ≈ IGF-1R	$1.51 \pm 0.68$ (8)	↑ IGF-1R
hCG (+)	$1.26 \pm 0.31$ (6)	↑ IGF-1R	$0.95 \pm 0.16$ (4)	IR ≈ IGF-1R	$1.34 \pm 0.55$ (8)	↑ IGF-1R
EGF (-)	$1.50 \pm 0.43$ (6)	↑ IGF-1R	$1.47 \pm 0.30$ (4)	↑ IGF-1R	$1.04 \pm 0.24$ (8)	IR ≈ IGF-1R
EGF (+)	$1.35 \pm 0.34$ (6)	↑ IGF-1R	$1.91 \pm 0.54$ (4)	↑ IGF-1R	$1.22 \pm 0.25$ (8)	↑ IGF-1R
IGF-1 (-)	$1.30 \pm 0.37$ (6)	↑ IGF-1R	$0.88 \pm 0.11$ (4)	↑ IR	$1.49 \pm 0.21$ (8)	↑ IGF-1R
IGF-1 (+)	$1.50 \pm 0.40$ (6)	↑ IGF-1R	$0.87 \pm 0.04$ (4)	↑ IR	$1.33 \pm 0.16$ (8)	↑ IGF-1R

**Table 3.2:** Significant differences in expression of *AMPK* subunits with **age**. The *AMPK* subunit gene is shown in the left column. Treatments producing significant differences between young and old animals or middle-aged and old animals, values for  $2^{-\Delta\Delta Ct}$  comparing young or middle-aged animals with older animals and p values are shown in the columns to the right.

Gene	Medium	Treatment	Age			Fold change ( $2^{-\Delta\Delta Ct}$ )	p
			Young $\Delta Ct \pm SE$	Middle $\Delta Ct \pm SE$	Old $\Delta Ct \pm SE$		
<i>AMPK <math>\alpha 1</math></i>	-	None	10.34 $\pm$ 1.21	-	7.76 $\pm$ 0.52	6.0	< 0.02
<i>AMPK <math>\alpha 1</math></i>	+	EGF	-	11.02 $\pm$ 1.01	8.46 $\pm$ 0.39	5.9	< 0.04
<i>AMPK <math>\beta 1</math></i>	-	None	-	10.50 $\pm$ 0.84	7.90 $\pm$ 0.71	6.1	< 0.03
<i>AMPK <math>\beta 1</math></i>	-	None	11.36 $\pm$ 1.03	-	7.90 $\pm$ 0.71	11.0	< 0.001
<i>AMPK <math>\beta 1</math></i>	-	Insulin	11.15 $\pm$ 0.83	-	7.77 $\pm$ 0.81	10.4	< 0.002
<i>AMPK <math>\beta 1</math></i>	+	EGF	-	11.71 $\pm$ 1.07	8.39 $\pm$ 0.66	10.0	< 0.005
<i>AMPK <math>\beta 1</math></i>	+	EGF	10.45 $\pm$ 0.64	-	8.39 $\pm$ 0.66	4.2	< 0.05
<i>AMPK <math>\beta 1</math></i>	-	P4	10.26 $\pm$ 0.50	-	8.09 $\pm$ 0.74	4.5	< 0.04
<i>AMPK <math>\gamma 3</math></i>	-	None	14.34 $\pm$ 0.88	-	11.31 $\pm$ 0.81	8.2	< 0.04
<i>AMPK <math>\gamma 3</math></i>	-	Insulin	-	13.77 $\pm$ 1.48	10.21 $\pm$ 1.12	11.8	< 0.03
<i>AMPK <math>\gamma 3</math></i>	+	hCG	-	14.43 $\pm$ 2.28	10.03 $\pm$ 0.63	21.1	< 0.01

**Table 3. 3:** Significant differences in expression of *AMPK* subunits with various **treatments**. The *AMPK* subunit gene is shown in the left column. Values for  $\Delta Ct$  for a specific treatment and values for  $2^{-\Delta\Delta Ct}$  between two treatments where there were significant differences are shown in the columns to the right.

Gene	Medium	Age	Treatment						Fold-change ( $2^{-\Delta\Delta Ct}$ )	p<
			None	Insulin	hCG	EGF	IGF-1	P4		
			$\Delta Ct \pm SE$	$\Delta Ct \pm SE$	$\Delta Ct \pm SE$	$\Delta Ct \pm SE$	$\Delta Ct \pm SE$	$\Delta Ct \pm SE$		
<i>AMPK<math>\alpha</math>1</i>	+	Middle		7.30±0.71		8.65±1.00			2.55	< 0.04
<i>AMPK<math>\alpha</math>1</i>	+	Middle		7.30±0.71				9.27±0.42	3.92	< 0.03
<i>AMPK<math>\alpha</math>2</i>	+	Middle		8.55±0.84	10.78±1.62				4.69	< 0.02
<i>AMPK<math>\alpha</math>2</i>	+	Middle		8.55±0.84		11.02±1.01			5.54	< 0.01
<i>AMPK<math>\alpha</math>2</i>	+	Middle		8.55±0.84				10.45±0.93	3.73	< 0.05
<i>AMPK<math>\alpha</math>2</i>	+	Old		9.81±0.62		8.46±0.39			0.39	< 0.05
<i>AMPK <math>\beta</math>1</i>	+	Middle	9.83±1.06			11.71±1.07			0.27	< 0.05
<i>AMPK <math>\beta</math>1</i>	+	Middle		8.35±0.47		11.71±1.07			0.10	< 0.004
<i>AMPK <math>\beta</math>1</i>	+	Middle		8.35±0.47				10.44±0.27	0.23	< 0.02
<i>AMPK <math>\beta</math>1</i>	+	Middle			9.56±1.17	11.71±1.07			0.23	< 0.02
<i>AMPK <math>\beta</math>1</i>	+	Middle				11.71±1.07	9.46±0.42		4.76	< 0.01
<i>AMPK <math>\beta</math>2</i>	+	Middle		7.20±0.53				9.43±0.74	4.69	< 0.02
<i>AMPK <math>\gamma</math>2</i>	+	Middle					12.83±1.51	15.03±2.62	0.22	< 0.04
<i>AMPK <math>\gamma</math>2</i>	+	Old	14.32±1.36			12.12±1.18			4.59	< 0.04
<i>AMPK <math>\gamma</math>3</i>	-	Old		10.21±1.12		13.31±1.28			0.12	< 0.001
<i>AMPK <math>\gamma</math>3</i>	-	Old			11.27±1.03	13.31±1.28			4.11	< 0.04
<i>AMPK <math>\gamma</math>3</i>	-	Old		10.21±1.12			12.32±0.77		0.23	< 0.03
<i>AMPK <math>\gamma</math>3</i>	-	Old				13.31±1.28		10.67±0.99	6.23	< 0.05
<i>AMPK <math>\gamma</math>3</i>	+	Old	12.27±1.03		10.03±0.63				0.21	< 0.02
<i>AMPK <math>\gamma</math>3</i>	+	Old			10.03±0.63	13.31±1.28			9.71	< 0.04
<i>AMPK <math>\gamma</math>3</i>	+	Old			10.03±0.63		12.62±0.86		0.17	< 0.02

**Table 3. 4:**  $\Delta\text{Ct}$  and fold change ( $2^{-\Delta\Delta\text{Ct}}$ ) in *IR* expression:

Age	Number	Treatment	Medium	$\Delta\text{Ct} \pm \text{SE}$	Mean Y $\pm$ SE Fold change	Mean ln Y $\pm$ SE Fold change	
Young	6	None	-	10.85 $\pm$ 1.16	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	
	6	Insulin	-	10.21 $\pm$ 0.71	3.25 $\pm$ 0.41	0.41 $\pm$ 0.63	
	6	hCG	-	10.84 $\pm$ 0.66	1.84 $\pm$ 0.77	0.00 $\pm$ 0.57	
	6	EGF	-	11.40 $\pm$ 0.80	1.26 $\pm$ 0.54	- 0.38 $\pm$ 0.56	
	6	IGF-1	-	10.94 $\pm$ 1.14	1.53 $\pm$ 0.62	- 0.07 $\pm$ 0.47	
	6	P4	-	10.69 $\pm$ 0.94	2.38 $\pm$ 1.15	0.11 $\pm$ 0.58	
	6	None	+	11.40 $\pm$ 0.66	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	
	6	Insulin	+	11.12 $\pm$ 0.88	2.66 $\pm$ 1.82	- 0.28 $\pm$ 0.81	
	6	hCG	+	11.51 $\pm$ 0.93	0.72 $\pm$ 0.16	- 0.55 $\pm$ 0.36	
	6	EGF	+	12.08 $\pm$ 0.83	0.55 $\pm$ 0.13	- 0.94 $\pm$ 0.48	
	6	IGF-1	+	12.06 $\pm$ 0.64	0.55 $\pm$ 0.11	- 0.93 $\pm$ 0.51	
	6	P4	+	12.37 $\pm$ 1.18	0.57 $\pm$ 0.21	- 1.15 $\pm$ 0.55	
	Middle	4	None	-	8.34 $\pm$ 0.44	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
		4	Insulin	-	9.20 $\pm$ 0.25	0.61 $\pm$ 0.14	- 0.59 $\pm$ 0.27
4		hCG	-	9.16 $\pm$ 0.28	0.70 $\pm$ 0.25	- 0.57 $\pm$ 0.37	
4		EGF	-	9.20 $\pm$ 0.61	0.78 $\pm$ 0.43	- 0.72 $\pm$ 0.59	
4		IGF-1	-	10.22 $\pm$ 0.83	0.42 $\pm$ 0.22	- 1.21 $\pm$ 0.46	
4		P4	-	8.84 $\pm$ 0.37	0.95 $\pm$ 0.35	- 0.34 $\pm$ 0.49	
4		None	+	10.16 $\pm$ 0.74	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	
4		Insulin	+	9.28 $\pm$ 0.59	1.95 $\pm$ 1.00	0.27 $\pm$ 0.49	
4		hCG	+	9.38 $\pm$ 0.75	5.15 $\pm$ 4.15	0.58 $\pm$ 0.83	
4		EGF	+	10.63 $\pm$ 1.12	1.10 $\pm$ 0.34	- 0.02 $\pm$ 0.27	
4		IGF-1	+	8.83 $\pm$ 0.39	2.15 $\pm$ 0.78	0.55 $\pm$ 0.39	
4		P4	+	10.33 $\pm$ 0.67	0.89 $\pm$ 0.89	- 0.12 $\pm$ 0.81	
Old		8	None	-	9.10 $\pm$ 0.60	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
		8	Insulin	-	8.19 $\pm$ 0.54	3.47 $\pm$ 1.73	0.63 $\pm$ 0.41 <sup>a</sup>
	8	hCG	-	10.11 $\pm$ 1.19	1.91 $\pm$ 1.04	- 0.70 $\pm$ 0.97 <sup>b</sup>	
	8	EGF	-	10.50 $\pm$ 1.29	2.89 $\pm$ 2.22	- 0.97 $\pm$ 1.08 <sup>b</sup>	
	8	IGF-1	-	10.22 $\pm$ 1.22	4.03 $\pm$ 3.23	- 0.78 $\pm$ 1.04 <sup>b</sup>	
	8	P4	-	11.08 $\pm$ 1.23	4.30 $\pm$ 3.57	- 0.68 $\pm$ 1.02 <sup>b</sup>	
	8	None	+	11.07 $\pm$ 1.33	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	
	7	Insulin	+	9.69 $\pm$ 0.60	1.88 $\pm$ 0.66	0.15 $\pm$ 0.45	
	8	hCG	+	9.02 $\pm$ 1.77	12.99 $\pm$ 10.37	0.69 $\pm$ 0.69	
	8	EGF	+	10.38 $\pm$ 1.56	12.47 $\pm$ 10.77	0.48 $\pm$ 0.70	
	8	IGF-1	+	11.33 $\pm$ 1.20	1.39 $\pm$ 0.53	- 0.18 $\pm$ 0.38	
	8	P4	+	10.29 $\pm$ 1.28	12.76 $\pm$ 7.87	0.54 $\pm$ 0.79	

<sup>a,b</sup> Values with different superscripts were ( $p < 0.001$ ) significantly different

**Table 3. 5:**  $\Delta$ Ct and fold change ( $2^{-\Delta\Delta Ct}$ ) in *IGF-1R* expression

Age	Number	Treatment	Medium	$\Delta$ Ct $\pm$ SE	Mean Y $\pm$ SE Fold change	Mean ln Y $\pm$ SE Fold change
Young	6	None	-	8.67 $\pm$ 0.98	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	6	Insulin	-	8.76 $\pm$ 1.45	2.41 $\pm$ 0.77	-0.09 $\pm$ 0.96
	6	hCG	-	9.61 $\pm$ 1.59	1.47 $\pm$ 0.53	-0.66 $\pm$ 0.94
	6	EGF	-	9.85 $\pm$ 1.50	1.19 $\pm$ 0.47	-0.82 $\pm$ 0.94
	6	IGF-1	-	10.50 $\pm$ 1.52	0.75 $\pm$ 0.29	-1.27 $\pm$ 0.89
	6	P4	-	8.62 $\pm$ 1.56	2.66 $\pm$ 1.29	0.03 $\pm$ 0.70
	6	None	+	8.78 $\pm$ 1.15	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	6	Insulin	+	9.98 $\pm$ 1.56	1.83 $\pm$ 1.02	-0.83 $\pm$ 0.92 <b>a</b>
	6	hCG	+	10.93 $\pm$ 1.49	0.51 $\pm$ 0.17	-1.49 $\pm$ 0.79 <b>a</b>
	6	EGF	+	10.45 $\pm$ 1.44	0.58 $\pm$ 0.16	-1.15 $\pm$ 0.69 <b>a</b>
	6	IGF-1	+	10.30 $\pm$ 1.72	1.11 $\pm$ 0.57	-1.05 $\pm$ 0.94 <b>a</b>
	6	P4	+	10.24 $\pm$ 1.49	0.67 $\pm$ 0.22	-1.01 $\pm$ 0.62 <b>a</b>
Middle	4	None	-	10.49 $\pm$ 0.30	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	4	Insulin	-	11.01 $\pm$ 0.93	1.05 $\pm$ 0.42	-0.36 $\pm$ 0.62
	4	hCG	-	9.59 $\pm$ 1.51	4.27 $\pm$ 1.67	0.62 $\pm$ 1.06
	4	EGF	-	9.64 $\pm$ 1.56	4.67 $\pm$ 2.86	0.45 $\pm$ 1.15
	4	IGF-1	-	11.87 $\pm$ 1.13	1.00 $\pm$ 0.59	-0.87 $\pm$ 0.92
	4	P4	-	11.23 $\pm$ 1.52	2.38 $\pm$ 1.82	-0.51 $\pm$ 1.16
	4	None	+	11.35 $\pm$ 0.66	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	4	Insulin	+	10.01 $\pm$ 1.39	2.43 $\pm$ 0.96	0.59 $\pm$ 0.48
	4	hCG	+	10.32 $\pm$ 1.24	4.27 $\pm$ 3.01	0.76 $\pm$ 0.64
	4	EGF	+	8.72 $\pm$ 0.93	10.44 $\pm$ 3.90	2.12 $\pm$ 0.40
	4	IGF-1	+	10.44 $\pm$ 1.13	1.74 $\pm$ 0.86	0.26 $\pm$ 0.42
	4	P4	+	11.24 $\pm$ 1.71	5.34 $\pm$ 4.81	0.08 $\pm$ 1.03
Old	8	None	-	10.45 $\pm$ 1.36	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	8	Insulin	-	10.29 $\pm$ 1.84	1.86 $\pm$ 0.71	0.18 $\pm$ 0.35
	8	hCG	-	10.28 $\pm$ 1.58	3.65 $\pm$ 1.39	-0.51 $\pm$ 1.09
	8	EGF	-	11.72 $\pm$ 1.74	28.17 $\pm$ 25.73	-0.81 $\pm$ 1.61
	8	IGF-1	-	10.28 $\pm$ 1.51	29.40 $\pm$ 27.10	0.18 $\pm$ 1.16
	8	P4	-	10.86 $\pm$ 1.34	14.57 $\pm$ 13.20	-0.22 $\pm$ 1.07
	8	None	+	12.42 $\pm$ 1.44	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	8	Insulin	+	11.40 $\pm$ 1.51	5.63 $\pm$ 2.90	0.71 $\pm$ 0.58 <b>b</b>
	8	hCG	+	9.97 $\pm$ 1.74	41.58 $\pm$ 1.62	1.03 $\pm$ 0.75 <b>b</b>
	8	EGF	+	9.65 $\pm$ 1.60	82.55 $\pm$ 1.82	1.29 $\pm$ 1.05 <b>b</b>
	8	IGF-1	+	11.51 $\pm$ 1.43	4.52 $\pm$ 2.35	0.63 $\pm$ 0.54 <b>b</b>
	8	P4	+	11.33 $\pm$ 1.51	32.0 $\pm$ 2.74	0.76 $\pm$ 0.83 <b>b</b>

<sup>a, b</sup> Significant difference between young and old age ( $p < 0.025$ ) in presence of +FBS/+glucose

**Table 3. 6:**  $\Delta$ Ct and fold change ( $2^{-\Delta\Delta Ct}$ ) in *GLUT4* expression

Age	Number	Treatment	Medium	$\Delta$ Ct $\pm$ SE	Mean Y $\pm$ SE Fold change	Mean ln Y $\pm$ SE Fold change
Young	6	None	-	16.55 $\pm$ 1.97	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	6	Insulin	-	17.03 $\pm$ 1.90	1.42 $\pm$ 0.69	-0.36 $\pm$ 0.64
	6	hCG	-	17.58 $\pm$ 2.21	1.70 $\pm$ 0.58	-0.71 $\pm$ 1.02
	6	EGF	-	17.94 $\pm$ 2.29	1.12 $\pm$ 0.47	-0.97 $\pm$ 0.86
	6	IGF-1	-	16.65 $\pm$ 1.83	2.43 $\pm$ 1.76	-0.07 $\pm$ 0.55
	6	P4	-	16.82 $\pm$ 1.95	3.33 $\pm$ 2.02	-0.18 $\pm$ 0.85
	6	None	+	16.50 $\pm$ 2.03	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	6	Insulin	+	18.87 $\pm$ 2.05	1.13 $\pm$ 0.97	-0.164 $\pm$ 0.82 <b>a</b>
	6	hCG	+	18.80 $\pm$ 2.17	0.49 $\pm$ 0.21	-1.59 $\pm$ 0.75 <b>a</b>
	6	EGF	+	18.48 $\pm$ 2.10	1.19 $\pm$ 0.94	-1.37 $\pm$ 0.86 <b>a</b>
	6	IGF-1	+	19.45 $\pm$ 2.25	0.33 $\pm$ 0.14	-2.04 $\pm$ 0.78 <b>a</b>
	6	P4	+	17.84 $\pm$ 1.93	0.58 $\pm$ 0.16	-0.92 $\pm$ 0.49 <b>a</b>
Middle	4	None	-	19.67 $\pm$ 0.47	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	4	Insulin	-	19.09 $\pm$ 1.07	3.39 $\pm$ 1.80	0.40 $\pm$ 0.90
	4	hCG	-	18.43 $\pm$ 1.16	5.68 $\pm$ 3.28	0.77 $\pm$ 1.03
	4	EGF	-	18.35 $\pm$ 1.39	5.91 $\pm$ 3.34	0.78 $\pm$ 1.10
	4	IGF-1	-	20.72 $\pm$ 0.57	0.86 $\pm$ 0.34	-0.64 $\pm$ 0.71
	4	P4	-	17.75 $\pm$ 1.16	7.60 $\pm$ 2.84	1.24 $\pm$ 1.04
	4	None	+	20.66 $\pm$ 0.29	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	4	Insulin	+	19.48 $\pm$ 1.35	2.67 $\pm$ 1.50	0.47 $\pm$ 0.59
	4	hCG	+	19.51 $\pm$ 0.83	4.71 $\pm$ 2.88	0.84 $\pm$ 0.73
	4	EGF	+	19.17 $\pm$ 0.82	5.59 $\pm$ 2.67	1.34 $\pm$ 0.52
	4	IGF-1	+	19.22 $\pm$ 0.87	4.11 $\pm$ 3.12	0.63 $\pm$ 0.67
	4	P4	+	20.45 $\pm$ 1.12	3.72 $\pm$ 2.93	0.14 $\pm$ 0.91
Old	8	None	-	17.04 $\pm$ 1.73	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	8	Insulin	-	16.84 $\pm$ 1.60	2.08 $\pm$ 0.71	0.14 $\pm$ 0.50
	8	hCG	-	17.17 $\pm$ 1.42	4.11 $\pm$ 2.00	-0.08 $\pm$ 0.81
	8	EGF	-	17.33 $\pm$ 1.38	4.62 $\pm$ 3.60	-0.20 $\pm$ 0.73
	8	IGF-1	-	17.70 $\pm$ 1.53	5.27 $\pm$ 4.40	-0.46 $\pm$ 0.83
	8	P4	-	17.27 $\pm$ 1.43	1.96 $\pm$ 1.12	-0.16 $\pm$ 0.47
	8	None	+	17.82 $\pm$ 1.72	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	8	Insulin	+	16.87 $\pm$ 1.35	15.86 $\pm$ 7.66	0.66 $\pm$ 1.02 <b>b</b>
	8	hCG	+	15.16 $\pm$ 1.68	90.75 $\pm$ 84.93	1.46 $\pm$ 0.95 <b>b</b>
	8	EGF	+	16.46 $\pm$ 1.56	230.55 $\pm$ 227.13	0.94 $\pm$ 1.08 <b>b</b>
	8	IGF-1	+	17.34 $\pm$ 1.51	8.03 $\pm$ 4.79	0.33 $\pm$ 0.94 <b>b</b>
	8	P4	+	17.61 $\pm$ 1.81	25.80 $\pm$ 24.33	0.14 $\pm$ 0.89 <b>b</b>

**a, b** Significant difference between young and old age ( $p < 0.05$ ) in presence of +FBS/+glucose

**Table 3. 7:**  $\Delta\text{Ct}$  and fold change ( $2^{-\Delta\Delta\text{Ct}}$ ) in *AMPK $\alpha$ 1* expression

Age	Number	Treatment	Medium	$\Delta\text{Ct} \pm \text{SE}$	Mean-Y $\pm$ SE ( $2^{-\Delta\Delta\text{Ct}}$ )	Mean ln Y $\pm$ SE ( $2^{-\Delta\Delta\text{Ct}}$ )
Young	6	None	-	$8.95 \pm 1.24$	$1.00 \pm 0.00$	$1.00 \pm 0.00$
	6	Insulin	-	$8.86 \pm 1.26$	$1.95 \pm 1.19$	$0.03 \pm 0.45$
	6	hCG	-	$8.54 \pm 1.07$	$1.57 \pm 0.19$	$0.28 \pm 0.25$
	6	EGF	-	$9.25 \pm 1.11$	$1.91 \pm 0.16$	$-0.21 \pm 0.23$
	6	IGF-1	-	$8.11 \pm 0.99$	$2.64 \pm 1.07$	$0.58 \pm 0.40$
	6	P4	-	$8.73 \pm 0.97$	$1.86 \pm 0.65$	$0.15 \pm 0.53$
	6	None	+	$9.53 \pm 1.51$	$1.00 \pm 0.00$	$1.00 \pm 0.00$
	6	Insulin	+	$8.29 \pm 0.98$	$1.73 \pm 0.67$	$0.18 \pm 0.45$
	6	hCG	+	$8.98 \pm 0.90$	$4.45 \pm 3.23$	$0.38 \pm 0.62$
	6	EGF	+	$8.65 \pm 1.00$	$6.56 \pm 5.60$	$0.55 \pm 0.60$
	6	IGF-1	+	$8.25 \pm 0.89$	$15.42 \pm 14.08$	$0.70 \pm 0.81$
	6	P4	+	$8.93 \pm 0.92$	$18.18 \pm 17.41$	$0.41 \pm 0.88$
Middle	4	None	-	$8.62 \pm 0.69$	$1.00 \pm 0.00$	$1.00 \pm 0.00$
	4	Insulin	-	$8.62 \pm 0.54$	$1.44 \pm 0.65$	$0.00 \pm 0.51$
	4	hCG	-	$8.43 \pm 0.19$	$1.66 \pm 0.89$	$0.14 \pm 0.47$
	4	EGF	-	$8.69 \pm 1.00$	$1.68 \pm 0.73$	$-0.17 \pm 0.88$
	4	IGF-1	-	$9.33 \pm 0.81$	$1.43 \pm 0.97$	$-0.41 \pm 0.74$
	4	P4	-	$8.12 \pm 0.45$	$1.74 \pm 0.51$	$0.35 \pm 0.42$
	4	None	+	$8.41 \pm 0.96$	$1.00 \pm 0.00$	$1.00 \pm 0.00$
	4	Insulin	+	$7.30 \pm 0.71$	$2.43 \pm 0.65$	$0.42 \pm 0.59$
	4	hCG	+	$8.90 \pm 1.25$	$0.96 \pm 0.89$	$-0.29 \pm 0.44$
	4	EGF	+	$9.75 \pm 0.67$	$0.76 \pm 0.73$	$-0.63 \pm 0.54$
	4	IGF-1	+	$8.22 \pm 0.61$	$0.97 \pm 0.97$	$-0.24 \pm 0.39$
	4	P4	+	$9.27 \pm 0.42$	$1.00 \pm 0.51$	$-0.59 \pm 0.67$
Old	8	None	-	$6.84 \pm 0.45$	$1.00 \pm 0.00$	$1.00 \pm 0.00$
	8	Insulin	-	$7.12 \pm 0.40$	$1.56 \pm 0.73$	$-0.20 \pm 0.44$
	8	hCG	-	$7.34 \pm 0.52$	$0.89 \pm 0.23$	$-0.35 \pm 0.26$
	8	EGF	-	$7.55 \pm 0.52$	$0.83 \pm 0.22$	$-0.50 \pm 0.32$
	8	IGF-1	-	$8.15 \pm 0.64$	$0.64 \pm 0.19$	$-0.91 \pm 0.41$
	8	P4	-	$7.02 \pm 0.65$	$1.77 \pm 0.89$	$-0.13 \pm 0.44$
	8	None	+	$7.53 \pm 0.50$	$1.00 \pm 0.00$	$1.00 \pm 0.00$
	7	Insulin	+	$7.60 \pm 0.50$	$3.50 \pm 2.56$	$-0.05 \pm 0.56$
	8	hCG	+	$7.83 \pm 0.81$	$2.78 \pm 2.07$	$-0.34 \pm 0.59$
	8	EGF	+	$7.60 \pm 0.46$	$1.62 \pm 0.83$	$-0.05 \pm 0.34$
	8	IGF-1	+	$8.54 \pm 0.74$	$0.92 \pm 0.31$	$-0.70 \pm 0.52$
	8	P4	+	$7.87 \pm 0.67$	$1.38 \pm 0.47$	$-0.23 \pm 0.48$

**Table 3. 8:**  $\Delta\text{Ct}$  and fold change ( $2^{-\Delta\Delta\text{Ct}}$ ) in *AMPK $\alpha$ 2* expression

Age	Number	Treatment	Medium	$\Delta\text{Ct} \pm \text{SE}$	Mean-Y $\pm$ SE ( $2^{-\Delta\Delta\text{Ct}}$ )	Mean ln Y $\pm$ SE ( $2^{-\Delta\Delta\text{Ct}}$ )
Young	6	None	-	10.34 $\pm$ 1.21	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	6	Insulin	-	10.37 $\pm$ 1.06	2.99 $\pm$ 2.18	-0.05 $\pm$ 0.71 <b>a</b>
	6	hCG	-	8.97 $\pm$ 1.08	3.14 $\pm$ 0.88	0.95 $\pm$ 0.28 <b>a</b>
	6	EGF	-	9.91 $\pm$ 0.92	1.77 $\pm$ 0.51	0.30 $\pm$ 0.37 <b>a</b>
	6	IGF-1	-	9.37 $\pm$ 0.92	2.71 $\pm$ 0.91	0.67 $\pm$ 0.38 <b>a</b>
	6	P4	-	9.36 $\pm$ 0.91	2.72 $\pm$ 0.79	0.68 $\pm$ 0.42 <b>a</b>
	6	None	+	10.98 $\pm$ 1.32	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
6	Insulin	+	9.76 $\pm$ 0.64	9.42 $\pm$ 5.82	0.85 $\pm$ 0.78	
6	hCG	+	9.91 $\pm$ 0.97	9.07 $\pm$ 6.49	0.75 $\pm$ 0.76	
6	EGF	+	9.92 $\pm$ 0.87	3.93 $\pm$ 1.84	0.74 $\pm$ 0.50	
6	IGF-1	+	9.72 $\pm$ 0.76	7.11 $\pm$ 4.04	0.88 $\pm$ 0.68	
6	P4	+	10.19 $\pm$ 0.82	5.68 $\pm$ 3.23	0.55 $\pm$ 0.71	
Middle	4	None	-	9.76 $\pm$ 1.04	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	4	Insulin	-	9.42 $\pm$ 0.19	2.24 $\pm$ 1.16	0.23 $\pm$ 0.68
	4	hCG	-	9.02 $\pm$ 0.67	2.02 $\pm$ 0.78	0.51 $\pm$ 0.35
	4	EGF	-	10.38 $\pm$ 1.47	0.83 $\pm$ 0.32	-0.56 $\pm$ 0.59
	4	IGF-1	-	10.41 $\pm$ 1.13	0.92 $\pm$ 0.34	-0.37 $\pm$ 0.46
	4	P4	-	9.58 $\pm$ 1.47	1.36 $\pm$ 0.45	0.12 $\pm$ 0.36
	4	None	+	9.26 $\pm$ 0.79	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
4	Insulin	+	8.55 $\pm$ 0.84	1.78 $\pm$ 0.78	0.15 $\pm$ 0.58	
4	hCG	+	10.78 $\pm$ 1.62	0.61 $\pm$ 0.25	-1.01 $\pm$ 0.74	
4	EGF	+	11.02 $\pm$ 1.01	0.89 $\pm$ 0.49	-0.92 $\pm$ 0.92	
4	IGF-1	+	9.32 $\pm$ 1.33	0.88 $\pm$ 0.39	-0.41 $\pm$ 0.43	
4	P4	+	10.45 $\pm$ 0.93	0.78 $\pm$ 0.45	-0.83 $\pm$ 0.67	
Old	8	None	-	7.76 $\pm$ 0.52	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	8	Insulin	-	8.45 $\pm$ 0.60	1.10 $\pm$ 0.37	-0.47 $\pm$ 0.48 <b>b</b>
	8	hCG	-	8.22 $\pm$ 0.39	0.91 $\pm$ 0.24	-0.32 $\pm$ 0.26 <b>b</b>
	8	EGF	-	8.27 $\pm$ 0.46	0.93 $\pm$ 0.27	-0.35 $\pm$ 0.28 <b>b</b>
	8	IGF-1	-	9.21 $\pm$ 0.49	0.41 $\pm$ 0.07	-1.00 $\pm$ 0.20 <b>b</b>
	8	P4	-	7.90 $\pm$ 0.45	3.57 $\pm$ 2.92	-0.09 $\pm$ 0.50 <b>b</b>
	8	None	+	9.18 $\pm$ 0.51	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
7	Insulin	+	9.81 $\pm$ 0.62	5.43 $\pm$ 4.60	-0.44 $\pm$ 0.76	
8	hCG	+	8.95 $\pm$ 0.43	3.34 $\pm$ 2.44	0.14 $\pm$ 0.47	
8	EGF	+	8.46 $\pm$ 0.39	4.03 $\pm$ 2.61	0.50 $\pm$ 0.46	
8	IGF-1	+	9.59 $\pm$ 0.56	1.49 $\pm$ 0.75	-0.29 $\pm$ 0.44	
8	P4	+	9.05 $\pm$ 0.72	2.30 $\pm$ 0.82	0.09 $\pm$ 0.57	

<sup>a, b</sup> Significant difference between young and old age ( $p < 0.027$ )

**Table 3. 9:**  $\Delta\text{Ct}$  and fold change ( $2^{-\Delta\Delta\text{Ct}}$ ) in *AMPK $\beta$ 1* expression

Age	Number	Treatment	Medium	$\Delta\text{Ct} \pm \text{SE}$	Mean-Y $\pm$ SE ( $2^{-\Delta\Delta\text{Ct}}$ )	Mean ln Y $\pm$ SE ( $2^{-\Delta\Delta\text{Ct}}$ )	
Young	6	None	-	11.36 $\pm$ 1.03 <b>a</b>	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	
	6	Insulin	-	11.15 $\pm$ 0.83 <b>a</b>	5.38 $\pm$ 4.06	0.12 $\pm$ 0.86	
	6	hCG	-	11.08 $\pm$ 0.95 <b>a</b>	4.05 $\pm$ 2.03	0.89 $\pm$ 0.42	
	6	EGF	-	10.55 $\pm$ 0.47 <b>a</b>	2.52 $\pm$ 0.75	0.56 $\pm$ 0.45	
	6	IGF-1	-	10.61 $\pm$ 0.43 <b>a</b>	2.77 $\pm$ 0.73	0.52 $\pm$ 0.61	
	6	P4	-	10.26 $\pm$ 0.50 <b>a</b>	3.32 $\pm$ 1.23	0.76 $\pm$ 0.47	
	6	None	+	11.12 $\pm$ 1.16	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	
	6	Insulin	+	10.30 $\pm$ 0.72	7.65 $\pm$ 6.64	0.57 $\pm$ 0.65	
	6	hCG	+	10.01 $\pm$ 0.85	6.88 $\pm$ 4.92	0.77 $\pm$ 0.64	
	6	EGF	+	10.45 $\pm$ 0.64	5.44 $\pm$ 4.49	0.46 $\pm$ 0.59	
	6	IGF-1	+	10.53 $\pm$ 0.50	5.14 $\pm$ 4.12	0.40 $\pm$ 0.62	
	6	P4	+	11.09 $\pm$ 0.65	8.66 $\pm$ 8.09	0.02 $\pm$ 0.82	
	Middle	4	None	-	10.50 $\pm$ 0.84	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
		4	Insulin	-	9.66 $\pm$ 0.85	2.69 $\pm$ 1.16	0.58 $\pm$ 0.56
4		hCG	-	9.69 $\pm$ 0.33	2.53 $\pm$ 1.33	0.56 $\pm$ 0.48	
4		EGF	-	9.91 $\pm$ 1.01	3.25 $\pm$ 1.55	0.28 $\pm$ 1.07	
4		IGF-1	-	10.80 $\pm$ 0.74	1.41 $\pm$ 0.79	-0.13 $\pm$ 0.56	
4		P4	-	9.46 $\pm$ 0.43	2.17 $\pm$ 1.25	0.28 $\pm$ 0.58	
4		None	+	9.83 $\pm$ 1.06	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	
4		Insulin	+	8.35 $\pm$ 0.47	2.55 $\pm$ 0.95	0.69 $\pm$ 0.42	
4		hCG	+	9.56 $\pm$ 1.17	1.71 $\pm$ 0.79	0.24 $\pm$ 0.45	
4		EGF	+	11.71 $\pm$ 1.07	0.78 $\pm$ 0.46	-1.00 $\pm$ 0.84	
4		IGF-1	+	9.46 $\pm$ 0.42	1.80 $\pm$ 1.20	-0.11 $\pm$ 0.70	
4		P4	+	10.44 $\pm$ 0.27	1.25 $\pm$ 0.70	-0.42 $\pm$ 0.71	
Old		8	None	-	7.90 $\pm$ 0.71 <b>b</b>	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
		8	Insulin	-	7.77 $\pm$ 0.81 <b>b</b>	2.23 $\pm$ 1.00	0.09 $\pm$ 0.52
	8	hCG	-	8.27 $\pm$ 0.69 <b>b</b>	1.15 $\pm$ 0.33	-0.26 $\pm$ 0.37	
	8	EGF	-	8.55 $\pm$ 0.66 <b>b</b>	0.92 $\pm$ 0.22	-0.46 $\pm$ 0.38	
	8	IGF-1	-	8.77 $\pm$ 0.70 <b>b</b>	0.83 $\pm$ 0.23	-0.61 $\pm$ 0.40	
	8	P4	-	8.09 $\pm$ 0.74 <b>b</b>	2.94 $\pm$ 1.79	-0.13 $\pm$ 0.61	
	8	None	+	9.07 $\pm$ 0.67	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	
	7	Insulin	+	9.18 $\pm$ 0.45	5.16 $\pm$ 4.01	-0.08 $\pm$ 0.65	
	8	hCG	+	9.00 $\pm$ 0.76	2.92 $\pm$ 2.15	-0.06 $\pm$ 0.50	
	8	EGF	+	8.39 $\pm$ 0.66	3.85 $\pm$ 2.37	0.47 $\pm$ 0.45	
	8	IGF-1	+	9.54 $\pm$ 0.83	1.83 $\pm$ 1.13	-0.32 $\pm$ 0.49	
	8	P4	+	9.12 $\pm$ 0.75	1.95 $\pm$ 0.88	-0.03 $\pm$ 0.49	

**a, b** Significant difference between young and age ( $p < 0.006$ ) in -FBS/-glucose for all treatments.

**Table 3. 10:**  $\Delta$ Ct and fold change ( $2^{-\Delta\Delta Ct}$ ) in AMPK $\beta$ 2 expression

Age	Number	Treatment	Medium	$\Delta$ Ct $\pm$ SE	Mean-Y $\pm$ SE ( $2^{-\Delta\Delta Ct}$ )	Mean ln Y $\pm$ SE ( $2^{-\Delta\Delta Ct}$ )
Young	6	None	-	8.50 $\pm$ 0.50	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	6	Insulin	-	8.31 $\pm$ 0.51	1.58 $\pm$ 0.66	0.11 $\pm$ 0.36
	6	hCG	-	8.48 $\pm$ 0.47	1.36 $\pm$ 0.53	0.02 $\pm$ 0.32
	6	EGF	-	8.64 $\pm$ 0.26	1.20 $\pm$ 0.39	-0.09 $\pm$ 0.34
	6	IGF-1	-	8.97 $\pm$ 0.40	1.63 $\pm$ 0.32	0.37 $\pm$ 0.23
	6	P4	-	7.97 $\pm$ 0.39	1.97 $\pm$ 0.73	0.37 $\pm$ 0.35
	6	None	+	9.15 $\pm$ 0.92	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	6	Insulin	+	8.56 $\pm$ 0.68	8.20 $\pm$ 0.41	0.41 $\pm$ 0.75
	6	hCG	+	8.41 $\pm$ 0.37	5.00 $\pm$ 0.52	0.52 $\pm$ 0.67
	6	EGF	+	8.45 $\pm$ 0.37	5.13 $\pm$ 0.49	0.49 $\pm$ 0.63
	6	IGF-1	+	8.32 $\pm$ 0.32	5.57 $\pm$ 0.58	0.58 $\pm$ 0.70
	6	P4	+	8.15 $\pm$ 0.39	9.54 $\pm$ 0.69	0.69 $\pm$ 0.83
Middle	4	None	-	8.09 $\pm$ 0.52	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	4	Insulin	-	8.39 $\pm$ 0.47	0.94 $\pm$ 0.28	0.21 $\pm$ 0.32
	4	hCG	-	7.84 $\pm$ 0.36	1.27 $\pm$ 0.26	0.17 $\pm$ 0.22
	4	EGF	-	7.82 $\pm$ 0.56	1.32 $\pm$ 0.46	0.06 $\pm$ 0.40
	4	IGF-1	-	9.13 $\pm$ 0.69	0.61 $\pm$ 0.16	0.64 $\pm$ 0.34
	4	P4	-	8.14 $\pm$ 0.46	1.06 $\pm$ 0.23	-0.03 $\pm$ 0.26
	4	None	+	8.07 $\pm$ 0.64	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	4	Insulin	+	7.20 $\pm$ 0.53	1.40 $\pm$ 0.32	0.26 $\pm$ 0.23
	4	hCG	+	8.59 $\pm$ 1.02	0.93 $\pm$ 0.31	0.32 $\pm$ 0.44
	4	EGF	+	9.03 $\pm$ 0.80	0.85 $\pm$ 0.26	0.37 $\pm$ 0.41
	4	IGF-1	+	7.98 $\pm$ 0.37	0.85 $\pm$ 0.27	0.31 $\pm$ 0.32
	4	P4	+	9.43 $\pm$ 0.74	0.47 $\pm$ 0.15	-0.95 $\pm$ 0.38
Old	8	None	-	7.70 $\pm$ 0.64	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	8	Insulin	-	7.40 $\pm$ 0.76	2.79 $\pm$ 1.44	0.21 $\pm$ 0.49
	8	hCG	-	8.44 $\pm$ 0.73	1.53 $\pm$ 0.91	-0.51 $\pm$ 0.53
	8	EGF	-	8.00 $\pm$ 0.62	1.18 $\pm$ 0.37	-0.21 $\pm$ 0.35
	8	IGF-1	-	7.73 $\pm$ 0.69	2.42 $\pm$ 1.30	-0.02 $\pm$ 0.48
	8	P4	-	7.54 $\pm$ 0.66	2.54 $\pm$ 1.49	0.12 $\pm$ 0.46
	8	None	+	7.94 $\pm$ 0.66	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	7	Insulin	+	8.72 $\pm$ 1.11	3.59 $\pm$ 1.95	-0.54 $\pm$ 1.06
	8	hCG	+	8.41 $\pm$ 0.47	1.25 $\pm$ 0.54	-0.29 $\pm$ 0.39
	8	EGF	+	7.93 $\pm$ 0.54	1.36 $\pm$ 0.44	0.00 $\pm$ 0.28
	8	IGF-1	+	8.29 $\pm$ 0.70	0.98 $\pm$ 0.20	-0.24 $\pm$ 0.29
	8	P4	+	7.85 $\pm$ 0.96	2.13 $\pm$ 1.01	0.06 $\pm$ 0.47

**Table 3. 11:**  $\Delta\text{Ct}$  and fold change ( $2^{-\Delta\Delta\text{Ct}}$ ) in *AMPK $\gamma$ 1* expression

Age	Number	Treatment	Medium	$\Delta\text{Ct} \pm \text{SE}$	Mean-Y $\pm$ SE ( $2^{-\Delta\Delta\text{Ct}}$ )	Mean ln Y $\pm$ SE ( $2^{-\Delta\Delta\text{Ct}}$ )
Young	6	None	-	8.10 $\pm$ 0.91	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	6	Insulin	-	8.36 $\pm$ 1.60	1.89 $\pm$ 0.64	-0.21 $\pm$ 0.88
	6	hCG	-	9.04 $\pm$ 1.94	1.32 $\pm$ 0.41	-0.66 $\pm$ 0.98
	6	EGF	-	9.08 $\pm$ 1.77	1.42 $\pm$ 0.58	-0.68 $\pm$ 0.95
	6	IGF-1	-	8.29 $\pm$ 1.61	1.95 $\pm$ 0.62	-0.13 $\pm$ 0.85
	6	P4	-	7.77 $\pm$ 1.83	2.67 $\pm$ 0.65	0.23 $\pm$ 0.89
	6	None	+	8.05 $\pm$ 0.73	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
6	Insulin	+	8.50 $\pm$ 1.56	1.41 $\pm$ 0.42	-0.31 $\pm$ 0.74	
6	hCG	+	9.17 $\pm$ 1.60	0.87 $\pm$ 0.23	-0.78 $\pm$ 0.75	
6	EGF	+	8.88 $\pm$ 1.71	1.33 $\pm$ 0.51	-0.57 $\pm$ 0.86	
6	IGF-1	+	9.34 $\pm$ 1.75	0.92 $\pm$ 0.29	-0.89 $\pm$ 0.89	
6	P4	+	8.68 $\pm$ 1.29	1.28 $\pm$ 0.54	-0.43 $\pm$ 0.61	
Middle	4	None	-	10.00 $\pm$ 1.06	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	4	Insulin	-	8.52 $\pm$ 0.57	4.17 $\pm$ 3.39	0.63 $\pm$ 0.89
	4	hCG	-	8.14 $\pm$ 0.22	3.77 $\pm$ 2.29	0.80 $\pm$ 0.80
	4	EGF	-	8.36 $\pm$ 0.28	3.58 $\pm$ 2.18	0.56 $\pm$ 1.02
	4	IGF-1	-	8.79 $\pm$ 0.50	3.03 $\pm$ 2.12	0.56 $\pm$ 0.74
	4	P4	-	8.52 $\pm$ 0.16	3.19 $\pm$ 1.97	0.55 $\pm$ 0.89
	4	None	+	8.90 $\pm$ 1.04	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
4	Insulin	+	7.78 $\pm$ 0.14	0.93 $\pm$ 0.31	0.22 $\pm$ 0.42	
4	hCG	+	7.69 $\pm$ 0.40	2.08 $\pm$ 0.95	0.52 $\pm$ 0.46	
4	EGF	+	8.94 $\pm$ 0.37	1.96 $\pm$ 1.52	-0.03 $\pm$ 0.83	
4	IGF-1	+	7.76 $\pm$ 0.03	0.98 $\pm$ 0.31	-0.18 $\pm$ 0.43	
4	P4	+	9.26 $\pm$ 0.56	0.50 $\pm$ 0.18	-0.81 $\pm$ 0.33	
Old	8	None	-	10.62 $\pm$ 1.61	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	8	Insulin	-	9.33 $\pm$ 1.75	4.21 $\pm$ 2.33	0.58 $\pm$ 0.61
	8	hCG	-	9.56 $\pm$ 1.51	6.01 $\pm$ 3.59	0.67 $\pm$ 0.72
	8	EGF	-	9.95 $\pm$ 1.34	2.63 $\pm$ 1.03	0.51 $\pm$ 0.46
	8	IGF-1	-	10.09 $\pm$ 1.14	3.54 $\pm$ 1.42	0.38 $\pm$ 0.74
	8	P4	-	9.62 $\pm$ 1.05	10.79 $\pm$ 6.25	0.72 $\pm$ 1.00
	8	None	+	10.56 $\pm$ 1.67	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
7	Insulin	+	10.53 $\pm$ 1.65	3.11 $\pm$ 1.59	0.13 $\pm$ 0.78	
8	hCG	+	9.34 $\pm$ 0.95	12.70 $\pm$ 9.88	1.17 $\pm$ 0.69	
8	EGF	+	9.81 $\pm$ 1.11	22.52 $\pm$ 17.86	1.10 $\pm$ 0.98	
8	IGF-1	+	10.56 $\pm$ 1.46	2.50 $\pm$ 0.71	0.73 $\pm$ 0.26	
8	P4	+	10.13 $\pm$ 1.11	17.25 $\pm$ 15.89	0.68 $\pm$ 0.87	

**Table 3. 12:**  $\Delta\text{Ct}$  and fold change ( $2^{-\Delta\Delta\text{Ct}}$ ) in *AMPK $\gamma$ 2* expression

Age	Number	Treatment	Medium	$\Delta\text{Ct} \pm \text{SE}$	Mean-Y $\pm$ SE ( $2^{-\Delta\Delta\text{Ct}}$ )	Mean ln Y $\pm$ SE ( $2^{-\Delta\Delta\text{Ct}}$ )	
Young	6	None	-	13.93 $\pm$ 1.87	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	
	6	Insulin	-	12.41 $\pm$ 1.42	4.70 $\pm$ 1.78	1.03 $\pm$ 0.52	
	6	hCG	-	11.60 $\pm$ 1.92	6.59 $\pm$ 2.37	1.62 $\pm$ 0.32	
	6	EGF	-	13.19 $\pm$ 1.45	4.43 $\pm$ 1.99	0.52 $\pm$ 0.77	
	6	IGF-1	-	11.80 $\pm$ 1.76	14.31 $\pm$ 10.91	1.48 $\pm$ 0.64	
	6	P4	-	13.26 $\pm$ 1.75	16.34 $\pm$ 14.06	0.47 $\pm$ 1.02	
	6	None	+	12.94 $\pm$ 1.59	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	
	6	Insulin	+	12.77 $\pm$ 1.78	5.00 $\pm$ 4.37	0.12 $\pm$ 0.65	
	6	hCG	+	11.35 $\pm$ 1.65	6.50 $\pm$ 3.00	1.06 $\pm$ 0.75	
	6	EGF	+	12.61 $\pm$ 1.73	5.28 $\pm$ 4.12	0.23 $\pm$ 0.74	
	6	IGF-1	+	12.40 $\pm$ 1.52	4.96 $\pm$ 3.42	0.37 $\pm$ 0.68	
	6	P4	+	12.80 $\pm$ 1.63	4.12 $\pm$ 3.13	0.10 $\pm$ 0.74	
	Middle	4	None	-	14.62 $\pm$ 1.69	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
		4	Insulin	-	15.04 $\pm$ 2.17	1.23 $\pm$ 0.69	-0.29 $\pm$ 0.59 <sup>a</sup>
4		hCG	-	14.39 $\pm$ 2.19	1.93 $\pm$ 0.22	0.65 $\pm$ 0.12	
4		EGF	-	12.24 $\pm$ 2.15	3.48 $\pm$ 1.55	0.76 $\pm$ 0.85 <sup>b</sup>	
4		IGF-1	-	13.83 $\pm$ 0.65	1.01 $\pm$ 0.42	-0.25 $\pm$ 0.57	
4		P4	-	12.09 $\pm$ 1.43	2.90 $\pm$ 1.01	0.95 $\pm$ 0.32 <sup>b</sup>	
4		None	+	15.61 $\pm$ 2.32	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	
4		Insulin	+	13.55 $\pm$ 2.51	6.70 $\pm$ 4.57	1.42 $\pm$ 0.67	
4		hCG	+	14.80 $\pm$ 2.40	1.96 $\pm$ 0.66	0.55 $\pm$ 0.36	
4		EGF	+	14.56 $\pm$ 1.32	2.45 $\pm$ 2.04	-0.08 $\pm$ 1.04	
4		IGF-1	+	12.83 $\pm$ 1.51	2.01 $\pm$ 0.97	-0.07 $\pm$ 1.15	
4		P4	+	15.03 $\pm$ 2.62	1.52 $\pm$ 1.48	-1.67 $\pm$ 1.59	
Old		8	None	-	13.22 $\pm$ 1.85	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
		8	Insulin	-	12.71 $\pm$ 1.79	1.68 $\pm$ 0.71	0.01 $\pm$ 0.42
	8	hCG	-	12.30 $\pm$ 1.23	13.64 $\pm$ 11.40	0.63 $\pm$ 0.87	
	8	EGF	-	12.59 $\pm$ 1.12	11.28 $\pm$ 9.88	0.25 $\pm$ 0.93	
	8	IGF-1	-	13.40 $\pm$ 0.70	25.75 $\pm$ 24.60	-0.22 $\pm$ 1.25	
	8	P4	-	12.35 $\pm$ 0.92	33.84 $\pm$ 22.99	0.70 $\pm$ 1.68	
	8	None	+	14.32 $\pm$ 1.36	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	
	7	Insulin	+	13.98 $\pm$ 1.09	2.56 $\pm$ 1.06	0.04 $\pm$ 0.66 <sup>a</sup>	
	8	hCG	+	12.98 $\pm$ 0.76	16.39 $\pm$ 12.15	1.19 $\pm$ 0.75	
	8	EGF	+	12.12 $\pm$ 1.18	54.48 $\pm$ 37.08	1.46 $\pm$ 1.08 <sup>b</sup>	
	8	IGF-1	+	13.38 $\pm$ 0.96	60.48 $\pm$ 56.79	0.64 $\pm$ 1.17	
	8	P4	+	12.55 $\pm$ 1.43	21.95 $\pm$ 19.06	1.84 $\pm$ 1.19 <sup>b</sup>	

<sup>a, b</sup> Significant difference between treatments ( $p < 0.05$ )

**Table 3. 13:**  $\Delta\text{Ct}$  and fold change ( $2^{-\Delta\Delta\text{Ct}}$ ) in *AMPK $\gamma$ 3* expression

Age	Number	Treatment	Medium	$\Delta\text{Ct} \pm \text{SE}$	Mean-Y $\pm$ SE ( $2^{-\Delta\Delta\text{Ct}}$ )	Mean ln Y $\pm$ SE ( $2^{-\Delta\Delta\text{Ct}}$ )
Young	6	None	-	14.34 $\pm$ 0.88	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	6	Insulin	-	12.28 $\pm$ 0.75	10.43 $\pm$ 6.48	1.40 $\pm$ 0.71
	6	hCG	-	12.94 $\pm$ 1.18	6.37 $\pm$ 2.80	0.97 $\pm$ 0.72
	6	EGF	-	13.82 $\pm$ 0.93	2.87 $\pm$ 1.21	0.36 $\pm$ 0.62
	6	IGF-1	-	14.23 $\pm$ 0.76	1.74 $\pm$ 0.62	0.07 $\pm$ 0.54
	6	P4	-	12.48 $\pm$ 1.04	12.92 $\pm$ 8.52	1.29 $\pm$ 0.90
	6	None	+	13.54 $\pm$ 0.51	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
6	Insulin	+	13.74 $\pm$ 0.65	2.27 $\pm$ 1.69	-0.14 $\pm$ 0.54	
6	hCG	+	12.72 $\pm$ 1.12	8.43 $\pm$ 7.02	0.57 $\pm$ 0.73	
6	EGF	+	14.06 $\pm$ 0.76	0.86 $\pm$ 0.20	-0.36 $\pm$ 0.32	
6	IGF-1	+	13.89 $\pm$ 0.73	0.91 $\pm$ 0.20	-0.24 $\pm$ 0.24	
6	P4	+	13.45 $\pm$ 1.03	3.75 $\pm$ 2.86	0.07 $\pm$ 0.72	
Middle	4	None	-	14.01 $\pm$ 1.06	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	4	Insulin	-	13.77 $\pm$ 1.48	1.37 $\pm$ 0.47	0.17 $\pm$ 0.30
	4	hCG	-	13.09 $\pm$ 1.84	3.38 $\pm$ 2.42	0.83 $\pm$ 0.92
	4	EGF	-	13.11 $\pm$ 2.00	3.82 $\pm$ 1.99	0.49 $\pm$ 0.87
	4	IGF-1	-	13.99 $\pm$ 1.56	2.01 $\pm$ 1.02	0.10 $\pm$ 0.70
	4	P4	-	12.83 $\pm$ 1.44	3.40 $\pm$ 1.47	0.82 $\pm$ 0.57
	4	None	+	13.88 $\pm$ 1.57	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
4	Insulin	+	12.71 $\pm$ 1.63	2.30 $\pm$ 1.11	0.46 $\pm$ 0.50	
4	hCG	+	14.43 $\pm$ 2.28	1.39 $\pm$ 0.84	-0.34 $\pm$ 0.73	
4	EGF	+	12.73 $\pm$ 1.23	3.68 $\pm$ 1.37	1.09 $\pm$ 0.38	
4	IGF-1	+	12.46 $\pm$ 1.72	2.53 $\pm$ 1.30	0.61 $\pm$ 0.43	
4	P4	+	13.27 $\pm$ 0.81	2.39 $\pm$ 1.26	0.42 $\pm$ 0.58	
Old	8	None	-	11.31 $\pm$ 0.81	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
	8	Insulin	-	*10.21 $\pm$ 1.12	4.51 $\pm$ 2.48	0.59 $\pm$ 0.54 <b>a</b>
	8	hCG	-	11.27 $\pm$ 0.66	2.47 $\pm$ 0.88	-0.14 $\pm$ 0.78
	8	EGF	-	*13.31 $\pm$ 1.28	1.70 $\pm$ 0.94	-1.55 $\pm$ 1.22 <b>b</b>
	8	IGF-1	-	*12.32 $\pm$ 0.77	1.98 $\pm$ 0.89	-0.86 $\pm$ 0.95 <b>b</b>
	8	P4	-	10.67 $\pm$ 0.99	23.28 $\pm$ 21.07	0.28 $\pm$ 1.05
	8	None	+	12.27 $\pm$ 1.03	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
7	Insulin	+	11.82 $\pm$ 0.91	8.63 $\pm$ 5.63	0.32 $\pm$ 0.93	
8	hCG	+	10.03 $\pm$ 0.63	49.59 $\pm$ 44.59	1.39 $\pm$ 0.83 <b>a</b>	
8	EGF	+	12.00 $\pm$ 0.67	5.78 $\pm$ 3.92	0.19 $\pm$ 0.81	
8	IGF-1	+	12.62 $\pm$ 0.86	2.09 $\pm$ 1.05	-0.24 $\pm$ 0.59 <b>b</b>	
8	P4	+	11.40 $\pm$ 1.30	114.39 $\pm$ 99.31	0.61 $\pm$ 1.25	

**a, b** Significant difference between treatments in old age ( $p < 0.01$ ).

**Table 3. 14:** Significant differences in expression of *AMPK* subunit genes. The specific gene shown in the left column treatments produced significant differences between young and old animals or middle aged and old animals together with (p values) are shown in the column to the right

Gene	Medium	Treatment	Age			Fold-change ( $2^{-\Delta\Delta Ct}$ )	p
			Young $\Delta Ct \pm SE$	Middle $\Delta Ct \pm SE$	Old $\Delta Ct \pm SE$		
<i>AMPK <math>\alpha 1</math></i>	-	None	8.95±1.24	-	6.95±0.45	4.35	< 0.05
<i>AMPK <math>\alpha 2</math></i>	-	None	10.34 ± 1.21	-	7.76 ± 0.52	6.0	< 0.02
<i>AMPK <math>\alpha 2</math></i>	+	EGF	-	11.02 ± 1.01	8.46 ± 0.39	5.9	< 0.04
<i>AMPK <math>\beta 1</math></i>	-	None	-	10.50 ± 0.84	7.90 ± 0.71	6.1	< 0.03
<i>AMPK <math>\beta 1</math></i>	-	None	11.36 ± 1.03	-	7.90 ± 0.71	11.0	< 0.001
<i>AMPK <math>\beta 1</math></i>	-	Insulin	11.15 ± 0.83	-	7.77 ± 0.81	10.4	< 0.002
<i>AMPK <math>\beta 1</math></i>	+	EGF	-	11.71 ± 1.07	8.39 ± 0.66	10.0	< 0.005
<i>AMPK <math>\beta 1</math></i>	+	EGF	10.45 ± 0.64	-	8.39 ± 0.66	4.2	< 0.05
<i>AMPK <math>\beta 1</math></i>	-	P4	10.26 ± 0.50	-	8.09 ± 0.74	4.5	< 0.04
<i>AMPK <math>\beta 1</math></i>	+	P4	11.09±0.65	-	9.23±0.75	3.63	< 0.05
<i>AMPK <math>\gamma 3</math></i>	-	None	14.34 ± 0.88	-	11.31 ± 0.81	8.2	< 0.04
<i>AMPK <math>\gamma 3</math></i>	-	Insulin	-	13.77 ± 1.48	10.21 ± 1.12	11.8	< 0.03
<i>AMPK <math>\gamma 3</math></i>	+	hCG	-	14.43 ± 2.28	10.03 ± 0.63	21.1	< 0.01

**Table 3. 15:** Significant differences in expression of *IR*, *IGF-1R*, and *GLUT4* with various treatments. The gene is shown in the left column. Values for  $\Delta\text{Ct}$  for a specific treatment and values for  $2^{-\Delta\Delta\text{Ct}}$  between two treatments where there were significant differences are shown in the columns to the right.

Gene	Medium	Age	Treatment						Fold-change ( $2^{-\Delta\Delta\text{Ct}}$ )	p
			None $\Delta\text{Ct} \pm \text{SE}$	Insulin $\Delta\text{Ct} \pm \text{SE}$	hCG $\Delta\text{Ct} \pm \text{SE}$	EGF $\Delta\text{Ct} \pm \text{SE}$	IGF-1 $\Delta\text{Ct} \pm \text{SE}$	P4 $\Delta\text{Ct} \pm \text{SE}$		
<i>IR</i>	-	Old		8.19±0.54	10.11±1.19				3.78	< 0.03
<i>IR</i>	-	Old		8.19±0.54		10.50±1.29			4.96	< 0.007
<i>IR</i>	-	Old		8.19±0.54				10.22±1.21	4.08	< 0.02
<i>IR</i>	-	Old		8.19±0.54				10.08±1.23	3.71	< 0.03
<i>IR</i>	+	Old	11.07±1.32		9.02±1.77				0.24	< 0.02
<i>IR</i>	+	Old			9.02±1.77			11.32±1.20	4.92	< 0.008
<i>IGF-1R</i>	+	Old	12.42±1.43		9.97±1.74				0.18	< 0.03
<i>IGF-1R</i>	+	Old	12.42±1.43			9.65±1.60			0.15	< 0.01
<i>GLUT4</i>	-	Middle					20.17±0.57	17.75±1.16	0.19	< 0.04
<i>GLUT4</i>	+	Old	17.82±1.27		15.16±1.68				0.16	< 0.007
<i>GLUT4</i>	+	Old			15.16±1.68		17.34±1.51		4.53	< 0.03
<i>GLUT4</i>	+	Old			15.16±1.68			17.61±1.80	5.46	< 0.01
<i>GLUT4</i>	+	Young	16.50±2.03	18.87±2.04					5.17	< 0.04
<i>GLUT4</i>	+	Young	16.50±2.03		18.80±2.17				4.92	< 0.04
<i>GLUT4</i>	+	Young	16.50±2.03				19.45±2.25		7.73	< 0.01

## CHAPTER 4: DISCUSSION

Results presented here suggest that assembly of insulin receptor homodimers is reduced in older animals, a result that would affect insulin responsiveness. Moreover, EGF increased GLUT4 expression over 200-fold in older animals while insulin and IGF-1 had more modest, albeit significant effects. Thus, in older animals, even in the presence of high circulating levels of glucose, insulin may have modest effects on glucose uptake into granulosa cells and availability of GLUT4 transporters in the membrane. Insulin resistance in older animals could therefore be a combination of several factors that include reductions in insulin receptor homodimers, insulin or decreased available GLUT4 transporters, any one of which would be sufficient to negatively impact insulin utilization by granulosa cells.

Our results are consistent with observations that insulin-resistance in mares is associated with aging and characterized by the impaired ability of insulin to stimulate glucose uptake into insulin sensitive tissues, particularly muscle and adipose tissue, in all species. Obesity may exacerbate insulin resistance associated with aging by placing additional stress on energy homeostatic mechanisms (80). Unger et al. (29) have suggested that fat accumulation as well as changes in the distribution of lipids may predispose an individual to insulin resistance. In horses, obesity, even over a duration of a few months, promotes insulin-resistance in healthy horses (81-83).

In addition to maintain insulin responsiveness, granulosa cells need to maintain IGF-1 responsiveness. The lack of IGF-1 receptor signaling in granulosa cells can increase apoptosis at all stages of follicle development and reduce responsiveness of secondary stage follicles to FSH

(16). Reduced FSH responsiveness prevents initiation of follicle development and subsequent estradiol production. Together, arrest of folliculogenesis can result with a loss of fertility.

Insulin-resistance alone is a serious metabolic disorder. However, other diseases associated with insulin resistance can affect overall animal health. As an example, laminitis in horses is a devastating clinical syndrome that may affect 15% of horses (84,85). Equine practitioners identify endocrine/metabolic issues as the most common causes of laminitis diagnosed in practice (86). In general, middle-aged horses are more susceptible to the development of endocrinopathic laminitis. Horses presented for laminitis at a veterinary hospital and diagnosed with endocrinopathic laminitis averaged 15 years of age, with two-thirds of the cases presenting with hyperinsulinemia and associated obesity (87). Vasoconstriction in the feet is ultimately believed to be a major contributor (86). As insulin has vasoregulatory properties, IRS could predispose horses to vasoconstriction through a series of events that reduce production of nitric oxide (NO) and the ability of blood vessels to handle vascular challenges (86). Administration of insulin to healthy horses will induce laminitis, indicating that there is a direct link to the hormone (88,89).

In humans, menopause may contribute to insulin resistance in women. Menopause is accompanied by the loss of ovarian estrogen production which, in turn, affects intra-abdominal fat deposition. Ovariectomy in rats causes weight gain and accumulation of adipose tissue in the abdomen (90), a result similar to that seen in estrogen receptor  $\alpha$  knockout mice (91,92). Insulin-resistance is also linked to polycystic ovary syndrome (PCOS) which is characterized by reduced insulin secretion and glucose intolerance. Because PCOS is quite common, a subset of women with PCOS also have Type II diabetes as well. Interestingly, insulin resistance in women with PCOS may be related to a genetic condition affecting insulin receptor signaling as well as an

enzyme involved in androgen biosynthesis, thus linking both insulin resistance and excess androgen biosynthesis seen in the syndrome.

A significant result of our studies is that *GLUT4* expression in these studies is decreased in middle aged and older animals compared to young animals following pre-incubation with or without FBS/glucose between middle and young mares ( $p < 0.09$ ) and between old and young mares ( $p < 0.05$ ). Glucose uptake, primarily by skeletal and cardiac muscle and adipose tissue, is a rate limiting step in glucose utilization by these tissues and is dependent on availability in the cell membrane of an insulin-responsive glucose transporter. In horses, as is the case of the other 10 ISRN Veterinary Science species, the insulin-responsive glucose transporter GLUT4 is impaired in skeletal muscle and adipose tissue during naturally occurring insulin resistance in horses. There is also evidence that decreased *GLUT4* expression in aging animals is linked to insulin resistance (93,94).

In cells pre-incubated with +FBS/+glucose, treatment with insulin, IGF-1, progesterone, hCG and EGF significantly increased expression of *GLUT4* with the largest effect seen in EGF-treated cells. This increased *GLUT4* expression presumably increased the likelihood of hormone-dependent glucose uptake by these cells if sufficient hormone-responsive insulin receptors were available. This may be problematic for granulosa cells from older animals exposed to high glucose if, as suggested in **Figure 3.2**, these cells are expressing fewer insulin receptor homodimers.

Although there have been comparatively few studies evaluating effects of insulin-resistance in granulosa cells, GLUT4 transporter function and subsequent nutrient deficiency in cells has been suggested as a contributor to reduced fertility in horses (95). Aging effects on gastrocnemius muscle glucose uptake, both under basal conditions and following insulin treatment, have been shown in rats where basal levels decreased about 37% and insulin-induced glucose

uptake was reduced 69% in older animals compared to young rats (53). These decreases in glucose uptake appeared to result from reduced expression of *GLUT4* transporter mRNA and GLUT4 protein. Whether effects on *GLUT4* expression are direct response to aging is not clear. The appearance of insulin-resistance linked to *GLUT4*, may also reflect long-standing the nutritional status of the animal. In pigs with mildly inadequate nutrition, (96) revealed that *GLUT4* was upregulated in their skeletal muscles with concurrent decrease in insulin sensitivity when compared with pig receiving twice daily nutrition intake. Negative energy balance selectively up-regulates muscle *GLUT4* gene expression, but conversely reduces insulin-dependent glucose uptake during postnatal development.

Age-related differences in the expression of *AMPK* genes suggests that there are significant differences in the metabolic activity of younger and older animals. This heterotrimeric enzyme is comprising of an  $\alpha$  catalytic subunit ( $\alpha 1$  or  $\alpha 2$ ), and  $\beta$  and  $\gamma$  regulatory subunit ( $\beta 1$ ,  $\beta 2$ ;  $\gamma 1$ ,  $\gamma 2$  or  $\gamma 3$ ) (reviewed in (97,98)). In skeletal muscle, activation of AMPK serves as an ATP and AMP sensor through the  $\gamma$  regulatory subunit. Active AMPK is important in pathways leading to ATP production via glucose metabolism, fatty acid oxidation and mitochondrial biosynthesis (34,99,100) while inhibiting anabolic pathways that use ATP (101,102). While effects of AMPK on metabolic function are reasonably well understood, there are, in contrast to results presented here, no clear effects of aging on *AMPK* gene expression. Qiang and coworkers examined expression of *AMPK $\alpha 1$*  and *AMPK $\alpha 2$*  mRNA and protein in the gastrocnemius muscle and found no effect of age in rats (53).

Finally, hCG treatment significantly increased expression of *IGF-1R* and *GLUT4*, but not *IR*, in granulosa cells from older animals pre-incubated in +FBS/+glucose medium. This result was somewhat surprising given reports of decreasing luteinizing hormone receptor number in older

mares. Sessions-Bresnahan and Carnevale (103) suggest that *LHR* levels are significantly reduced in older mares while *FSHR* levels are reduced but not significantly so. Incidentally, results for *LHR* differ from those reported for bovine granulosa cells where *LHR* levels were elevated (104). There are several experimental factors that may affect this result including the presence of growth factors in FBS or, less likely given the lack of hCG response in cells pre-incubated without FBS/glucose, the use of hCG to induce ovulation in these animals.

One difficulty with these data are the variable effects of aging on our results. At the beginning of this project, we made a decision to separate animals into three groups that were comprised of young, middle-aged and older animals. As discussed in Chapter 1, it is difficult to determine whether there is a single, specific age at which mares transition from being young to middle-aged and from middle-aged to old. Aging in mares, as in humans, is influenced by the genetic make-up of an individual and environmental conditions. In mares, metabolic disease which is often difficult to identify without extensive laboratory work, can occur at any age and becomes more prevalent as animals age. To both simplify data analysis and also to make differences between young and older animals more apparent, it would be appropriate to eliminate the separate group of middle-aged animals from our analysis or, alternatively, to reassign animals into two groups made up of young animals (<15 years old) and old animals (>15 years old).

Reanalyzing all of our data is beyond the scope of this dissertation. However, when we examine the ratio of *IR:IGF1R* expression as originally presented in **Table 3.1**, the following results shown in **Table 4.1** were obtained. Younger animals are predicted to express more IGF-1 receptor under control conditions, either -FBS/-glucose or +FBS/+glucose, and following treatment of cells with EGF, insulin, IGF-1 or hCG. Older animals, on the other hand, are predicted to express approximately equal numbers of insulin receptors, IGF-1 receptors and hybrid receptors

in the absence of FBS/30 nM glucose but demonstrate a marked increase in insulin receptor expression when exposed to high glucose. Thus, in the presence of FBS/glucose, older animals are likely to have more insulin receptors and, assuming that other components of the insulin signaling pathway remain available, more responsive to insulin.

The other effect of age is seen in EGF-treated cells where, in the absence of FBS/30 nM glucose, cells are predicted to express approximately equal numbers of insulin receptors, IGF-1 receptors and hybrid receptors. Interestingly, when FBS/30 nM glucose are present, the expression of *IGF-1R* increases markedly and this would be predicted to assembly of IGF-1 receptors and hybrid receptors at the expense of insulin receptor formation leading to decreased insulin responsiveness.

This reanalysis of available data point out another important result of this project. Insulin insensitivity is likely to be due to a number of factors including many factors that we have not identified in this project. As discussed in the next chapter, there are a number of approaches that would be appropriate at this point. Probably the most straight forward approach would be to analyze samples that we obtained and preserved when collecting the data presented in this dissertation. We made a decision to use only “complete” data sets from a given animal and to combine these data with “complete data sets” obtained from other animals of similar age. There are, however, incomplete data sets where some effects of treatments, as an example, are missing. Nonetheless, with appropriate statistical analysis, this data is usable. This additional data might clarify effects of aging with specific treatments.

**Table 4.1:** The ratio  $\Delta\text{Ct (IR)}$  to  $\Delta\text{Ct (IGF-1R)}$  for *individual* young mares less than 15 years old (n = 10 animals) and older animals greater than 15 years old (n = 8 animals). Shown are predicted effects of these ratios on expression of insulin receptor homodimers and IGF-1R homodimers. Note that, as  $\Delta\text{Ct}$  values *increase*, the amount of mRNA expressed *decreases*. Thus, ratios of  $\Delta\text{Ct (IR)}$  to  $\Delta\text{Ct (IGF-1R)} > 1$ , are predicted that IGF-1R monomers should be the predominant species expressed in granulosa cells and that these cells will contain increased numbers of IGF-1R and hybrid receptors formed from insulin and IGF-1R monomers. When ratios are  $< 1$ , insulin receptors will be the predominant receptors found on granulosa cells.

Treatment	Young Mares < 15 years		Older Mares > 15 years	
	$\Delta\text{Ct (IR)} / \Delta\text{Ct (IGF-1R)}$	interpretation	$\Delta\text{Ct (IR)} / \Delta\text{Ct (IGF-1R)}$	interpretation
Control (-)	1.16 ± 0.19 (10)	↑ IGF-1R	0.99 ± 0.19 (8)	IR ≈ IGF-1R
Control (+)	1.28 ± 0.24 (10)	↑ IGF-1R	0.89 ± 0.19 (8)	↑ IR
Insulin (-)	1.27 ± 0.32 (10)	↑ IGF-1R	1.22 ± 0.42 (8)	↑ IGF-1R
Insulin (+)	1.16 ± 0.17 (10)	↑ IGF-1R	1.29 ± 0.51 (8)	↑ IGF-1R
hCG (-)	1.31 ± 0.30 (10)	↑ IGF-1R	1.51 ± 0.68 (8)	↑ IGF-1R
hCG (+)	1.14 ± 0.20 (10)	↑ IGF-1R	1.34 ± 0.55 (8)	↑ IGF-1R
EGF (-)	1.29 ± 0.27 (10)	↑ IGF-1R	1.04 ± 0.24 (8)	IR ≈ IGF-1R
EGF (+)	1.35 ± 0.21 (10)	↑ IGF-1R	1.22 ± 0.25 (8)	↑ IGF-1R
IGF-1 (-)	1.13 ± 0.23 (10)	↑ IGF-1R	1.49 ± 0.21 (8)	↑ IGF-1R
IGF-1 (+)	1.25 ± 0.26 (10)	↑ IGF-1R	1.33 ± 0.16 (8)	↑ IGF-1R

## CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

### 5. 1: Conclusions

There are significant differences between young and old mares with respect to insulin responses, predicted expression of insulin and IGF-1 receptors and metabolic activity. These differences are summarized in **Tables 5.1 – 5.5**. As shown in **Table 5.1**, expression of *IGF-1R* is significantly reduced in older animals as indicated by an increase in  $\Delta C_t$  values from 8.8 in young animals to 12.4 in older animals. Such a decrease in *IGF-1R* expression without a significant change in *IR* expression would be likely to increase the availability of IR monomers in the endoplasmic reticulum and thus increase numbers of insulin receptors and hybrid receptors formed from insulin monomers. This predicted result is shown in **Table 3.1** for otherwise untreated granulosa cells preincubated with medium containing +FBS/+30 nM glucose.

Two things are notable here. First, this predicted increase in insulin receptor assembly occurs in the presence of +FBS/+30 nM glucose and is not seen when cells are incubated in -FBS/-glucose medium. Second, a similar response to the presence of +FBS/+30 nM glucose is not seen in younger animals where receptors formed from IGF-1R monomers are believed to be the predominant receptor species. To test whether predicted changes in receptor populations are significant, it would be necessary to measure the absolute numbers of insulin receptors, IGF-1 receptors and hybrid receptors present on the cell membrane of granulosa cells. This is discussed below in future studies.

Also shown in **Table 5.1** are changes in AMPK subunit expression in medium lacking FBS and glucose. For each of the subunits shown, there is a 4.2 - 11.0 fold increase in AMPK subunit expression in older animals compared to younger animals that is significant. These subunits are both regulatory and catalytic subunits. This suggests that these granulosa cells from older animals are more metabolically active in the absence of FBS/30 nM glucose. Insulin treatment similarly increases *AMPK $\beta$ 1* expression.

**Tables 5.2 and 5.3** provide details for significant differences between *AMPK $\alpha$ 2* and *AMPK 1 $\beta$*  expression in young and older animals when granulosa cells are pre-incubated in medium lacking FBS/30 nM glucose. These data, as discussed in Chapter 3 were evaluated collectively and showed that there was a significant difference in age for both untreated and treated cells, regardless of treatment. In the presence of FBS/30 nM glucose, *IGF-1R* and *GLUT4* exhibited increased expression in granulosa cells from older animals pre-incubated in +FBS/+30 nM glucose and then treated with various hormones (**Tables 5.4 and 5.5**). Together these results support the idea that older mares are more labile with respect to changes in cell environment compared to younger animals. This affects both the metabolic activity of the cells as well as availability of intracellular glucose for catabolic metabolism. Whether these changes in gene expression reflect the development of insulin-resistance with aging would need to be assessed in individual animals using glucose tolerance tests and body scoring together with gene expression studies.

## 5. 2: Future directions

Reduced fertility in obese mares (105) suggests that long-term metabolic status may affect *IR* in sub-fertile animals. Our preliminary results suggest that increased expression of *IGF-1R* relative to *IR* may reduce the numbers of  $IR_2$  homodimers and increase both  $IGF1R_2$  homodimers and formation of *IR*-*IGF-1R* hybrid receptors in young animals while older animals are more environmentally- or hormone- sensitive with respect to  $IR_2$  and  $IGF-1R_2$  expression. The goal of future studies would be to clarify whether the environmental sensitivity of granulosa cells in older mares is due to the presence of growth factors as found, for example in FBS, extracellular glucose or to hormonal effects such as effects of follicle stimulating hormone (FSH) (106).

To study the effects of pre-incubation media, we would be interested in times immediately following isolation of granulosa cells from mares. At that time, the effects of media, *i.e.* low or high glucose, the presence of FBS providing a growth factor-rich medium or a combination of high glucose and FBS, could be assessed. The responsiveness of these granulosa cells to hormone exposure for 24 or 48 hr treatment on gene expression by these cells would then be examined. These experiments are shown schematically in **Figure 5.1**.

After isolating granulosa cells, we would then evaluate levels of genes for *IR*, *IGF-1R*, *AMP kinase* subunits as described above and *GLUT4*. In addition, we would examine levels of genes for *FSH receptor (FSHR)* and *LH receptors (LHR)* as described by Sessions-Bresnahan and Carnevale (103). Their results suggest that *LHR* levels are significantly reduced in older mares while *FSHR* levels are reduced but not significantly so. Incidentally, results for *LHR* differ from those reported for bovine granulosa cells where *LHR* levels were elevated (104). At the same time, we would begin an incubation of these cells in medium containing either +FBS or +30 nM glucose alone or +FBS/+30 nM glucose.

Cells would be harvested at 1 hr and at 24 hrs and gene expression will be evaluated for the genes described above. Finally, we would look in more detail at effects of 24 to 48 hr treatments with insulin, P4, IGF-1, FSH, hCG, IGF-1 and EGF.

In conjunction with these experiments, cell aliquots would be tested for cell proliferation using a commercial MTT assay (107). Baranao and Hammond (108) report that porcine granulosa cells are differentially sensitive to IGF-1 > insulin with respect to cell proliferation although animal age was not considered as a factor in these studies. IGF-1 is also reported to increase numbers of granulosa cells in bovine granulosa cells (109). Dupont and Scaramuzzi (106) suggest that FSH- and LH-mediated signaling may interact with insulin signaling although the consequences of such interactions on granulosa cells are not known.

To better understand the role of insulin receptors and IGF-1R in granulosa cell signaling, it is also important to understand what receptor species are involved in signaling, where they are located laterally in the membrane and how the magnitude of hormone-induced signals relates to receptor expression and actual receptor surface densities. Fluorescence correlation spectroscopy (FCS) makes possible direct measurements of molecular density for molecular species on individual living cells and has been applied to studies enumerating insulin receptors and IGF-1R on single cells (110). In FCS a low-power laser beam is focused on a cell surface and fluorescence from the molecule(s) of interest are measured over time using single-photon counting. As fluorescent molecules diffuse in and out of the laser spot, the fluorescence signal fluctuates. The time-scale of these fluctuations reflects the diffusion constant of the molecules. The number of molecules in the illuminated spot is contained in the time-zero amplitude of an appropriate auto- or cross-correlation function  $g(\tau)$  of signal(s) from detector(s) measuring a particular molecule's fluorescence  $F$ . As an example, counting LHR involves measuring  $g(0)$

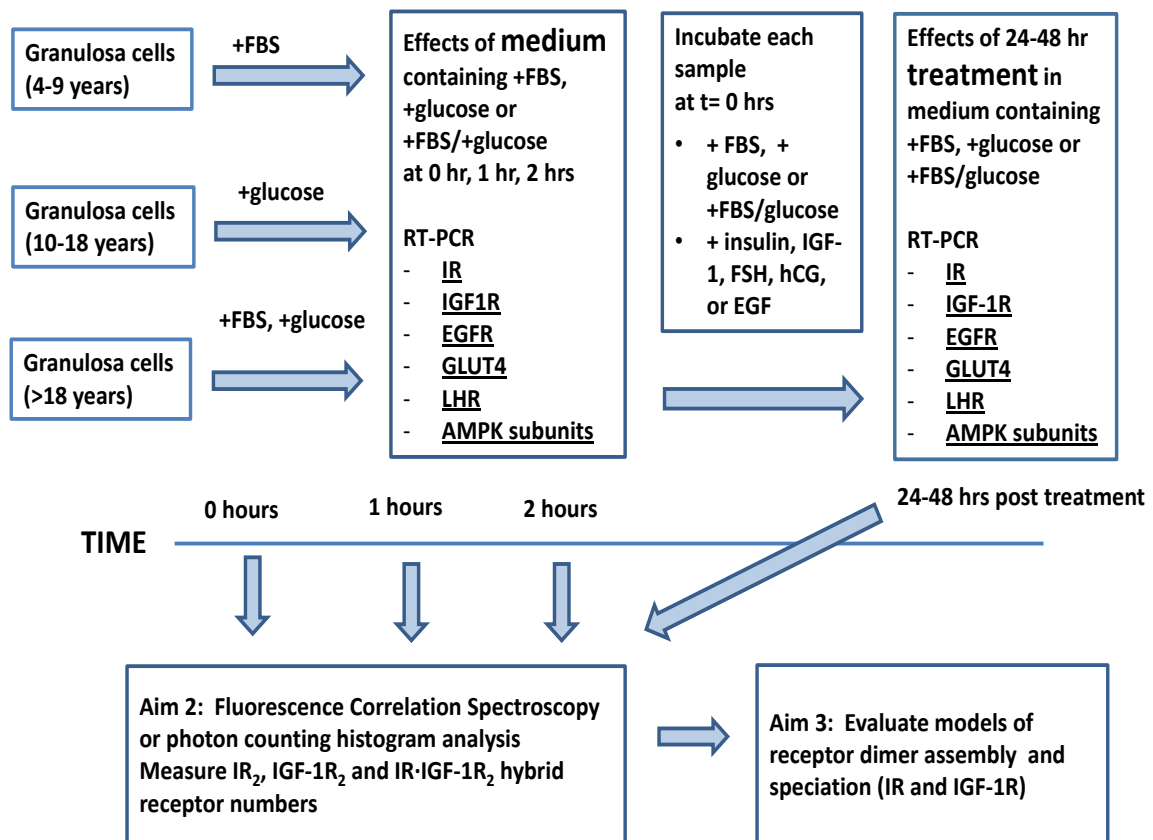
$$= \langle F_{LHR}^2 \rangle / (\langle F_{LHR} \rangle^2) - 1$$

where brackets denote time-averages of data. The receptor surface density  $\sigma$  is then calculated as  $\sigma = 2 / (\pi r_0^2 g(0))$ . The only required calibration parameter is the laser spot size  $r_0$  which is known from optical system parameters and verified from measured fluorescent dye diffusion rates.

A related method, photon counting histogram (PCH) analysis, provides complementary information on molecular sizes and speciation (111). PCH analysis is performed on fluorescence intensities measured by individual photon counting as in FCS experiments. When a laser beam is focused on a cell surface, the number of photons  $n$  detected within a measurement interval  $\Delta t$  from an annulus  $i$  around the beam having area  $dA_i$  and intensity  $B_i$  fluctuates not only because of diffusion-caused fluctuations in the number of *particles* in the laser beam, but also because of Poisson fluctuations in the number of *photons* emitted and detected from each particle. The photon counting histogram (PCH) curve shape  $P$ , a double Poisson distribution, reflects the surface densities  $\sigma$  of molecular aggregates of various sizes  $m$  and the rate of detected photon emission per molecule, the so-called molecular brightness  $q$ . Besides providing an alternative measurement of molecular surface densities, PCH analysis provides a direct measure of the aggregate size of a diffusing species since its molecular brightness can be compared to that of a known monomer. For example, we have used PCH analysis to examine possible multivalent binding of insulin to insulin receptors (110) and hormone-induced clustering of LHR (112).

Together these studies will establish in small numbers of cells or, in the case of fluorescence correlation spectroscopy and related methods, individual cells, the effect of cell environment on cell proliferation and numbers of receptors for insulin and IGF-1. These studies would represent an important methodological advance to understanding follicle function in the mare and, by extension, in humans. Moreover, single cell methods provide an opportunity to examine another critical cell in the pre-ovulatory follicle, the oocyte.

Information on the oocyte, together with information on granulosa cells that support oocyte development and function, would be invaluable to our understanding of similarities and differences in these cell types and suggest possible synergistic functions for these cells within the follicle. These studies, if conducted in younger animals and older animals would also be important in determining a time-course for follicular changes during aging and the possible consequences of aging on fertility.



**Figure 5.1:** Schematic representation of an experimental plan to evaluate the effects of granulosa cell medium on gene expression of insulin and IGF-1 receptor populations.

**Table 5. 1:** Comparison of young and older animals showing genes exhibiting significant differences in expression.

	Medium	Treatment	Age			
			Young $\Delta Ct \pm SE$	Old $\Delta Ct \pm SE$	$(2^{-\Delta\Delta Ct})$ for Fold- change	P
<i>AMPK <math>\alpha 1</math></i>	-	None	8.95±1.24	6.95±0.45	4.35	< 0.05
<i>AMPK <math>\alpha 2</math></i>	-	None	10.34 ± 1.21	7.76 ± 0.52	6.0	< 0.02
<i>AMPK <math>\beta 1</math></i>	-	None	11.36 ± 1.03	7.90 ± 0.71	11.0	< 0.001
<i>AMPK <math>\beta 1</math></i>	-	Insulin	11.15 ± 0.83	7.77 ± 0.81	10.4	< 0.002
<i>AMPK <math>\beta 1</math></i>	+	EGF	10.45 ± 0.64	8.39 ± 0.66	4.2	< 0.05
<i>AMPK <math>\gamma 3</math></i>	-	None	14.34 ± 0.88	11.31 ± 0.81	8.2	< 0.04
<i>IGF1R</i>	+	None	8.78±1.15	12.42±1.44	12.47	< 0.05

**Table 5.2:** Fold-change in expression of *AMPK  $\alpha$ 2* in older mares compared to younger mares

Gene	Medium	Treatment	Age			
			Young $\Delta$ Ct $\pm$ SE	Old $\Delta$ Ct $\pm$ SE	( $2^{-\Delta\Delta$ Ct) for Fold- change	P
<i>AMPK <math>\alpha</math>2</i>	-	None	10.34 $\pm$ 1.21	7.76 $\pm$ 0.52	3.98	< 0.027
<i>AMPK <math>\alpha</math>2</i>	-	Insulin	10.37 $\pm$ 1.06	8.45 $\pm$ 0.60	3.78	< 0.027
<i>AMPK <math>\alpha</math>2</i>	-	hCG	8.97 $\pm$ 1.08	8.22 $\pm$ 0.39	1.68	< 0.027
<i>AMPK <math>\alpha</math>2</i>	-	EGF	9.91 $\pm$ 0.92	8.27 $\pm$ 0.46	3.12	< 0.027
<i>AMPK <math>\alpha</math>2</i>	-	IGF-1	9.37 $\pm$ 0.92	9.21 $\pm$ 0.49	1.12	< 0.027
<i>AMPK <math>\alpha</math>2</i>	-	P4	9.36 $\pm$ 0.91	7.90 $\pm$ 0.45	2.75	< 0.027

**Table 5.3:** Fold-change in expression of *AMPK  $\beta$ 1* in older mares compared to younger mares

Gene	Medium	Treatment	Age			
			Young $\Delta\text{Ct} \pm \text{SE}$	Old $\Delta\text{Ct} \pm \text{SE}$	$(2^{-\Delta\Delta\text{Ct}})$ for Fold- change	P
<i>AMPK <math>\beta</math>1</i>	-	None	11.36 $\pm$ 1.03	7.90 $\pm$ 0.71	11	< 0.006
<i>AMPK <math>\beta</math>1</i>	-	Insulin	11.15 $\pm$ 0.83	7.77 $\pm$ 0.81	10.41	< 0.006
<i>AMPK <math>\beta</math>1</i>	-	hCG	11.08 $\pm$ 0.95	8.27 $\pm$ 0.69	7.01	< 0.006
<i>AMPK <math>\beta</math>1</i>	-	EGF	10.55 $\pm$ 0.47	8.55 $\pm$ 0.66	4.00	< 0.006
<i>AMPK <math>\beta</math>1</i>	-	IGF-1	10.61 $\pm$ 0.43	8.77 $\pm$ 0.70	3.58	< 0.006
<i>AMPK <math>\beta</math>1</i>	-	P4	10.26 $\pm$ 0.50	8.09 $\pm$ 0.74	4.50	< 0.006

**Table 5.4:** Fold-change in expression of *IGF-1R* in older mares compared to younger mares

Gene	Medium	Treatment	Age			
			Young $\Delta\text{Ct} \pm \text{SE}$	Old $\Delta\text{Ct} \pm \text{SE}$	$(2^{-\Delta\Delta\text{Ct}})$ for Fold- change	P
<i>IGF1R</i>	+	None	$8.78 \pm 1.15$	$12.42 \pm 1.44$	12.47	< 0.025
<i>IGF1R</i>	+	Insulin	$9.98 \pm 1.56$	$11.40 \pm 1.51$	2.68	< 0.025
<i>IGF1R</i>	+	hCG	$10.93 \pm 1.49$	$9.97 \pm 1.74$	0.51	< 0.025
<i>IGF1R</i>	+	EGF	$10.45 \pm 1.44$	$9.65 \pm 1.60$	0.57	< 0.025
<i>IGF1R</i>	+	IGF-1	$10.30 \pm 1.72$	$11.51 \pm 1.43$	2.31	< 0.025
<i>IGF1R</i>	+	P4	$10.24 \pm 1.49$	$11.33 \pm 1.51$	2.13	< 0.025

**Table 5.5:** Fold-change in expression of *GLUT4* in older mares compared to younger mares

Gene	Medium	Treatment	Age			
			Young $\Delta\text{Ct} \pm \text{SE}$	Old $\Delta\text{Ct} \pm \text{SE}$	$(2^{-\Delta\Delta\text{Ct}})$ for Fold- change	P
<i>GLUT4</i>	+	None	$16.50 \pm 2.03$	$17.82 \pm 1.72$	0.40	< 0.05
<i>GLUT4</i>	+	Insulin	$18.87 \pm 2.05$	$16.87 \pm 1.35$	4.00	< 0.05
<i>GLUT4</i>	+	hCG	$18.80 \pm 2.17$	$15.16 \pm 1.68$	12.47	< 0.05
<i>GLUT4</i>	+	EGF	$18.48 \pm 2.10$	$16.46 \pm 1.56$	4.06	< 0.05
<i>GLUT4</i>	+	IGF-1	$19.45 \pm 2.25$	$17.34 \pm 1.51$	4.32	< 0.05
<i>GLUT4</i>	+	P4	$17.84 \pm 1.93$	$17.61 \pm 1.81$	1.17	< 0.05

## REFERENCES

1. Kidder GM, Vanderhyden BC. Bidirectional communication between oocytes and follicle cells: ensuring oocyte developmental competence. *Canadian journal of physiology and pharmacology* 2010; 88:399-413
2. Carnevale EM. The mare model for follicular maturation and reproductive aging in the woman. *Theriogenology*  
Proceedings of the IETS Pre- and Post-Conference Symposia: - Animal Models of  
Reproduction/Recent Developments in the Practice of Bovine Embryo Transfer, IETS  
2008 Pre- and Post-Conference Symposia 2008; 69:23-30
3. Spicer LJ, Chamberlain CS, Maciel SM. Influence of gonadotropins on insulin- and insulin-like growth factor-I (IGF-I)-induced steroid production by bovine granulosa cells. *Domestic Animal Endocrinology* 2002; 22:237-254
4. Velazquez MA, Zaraza J, Oropeza A, Webb R, Niemann H. The role of IGF1 in the in vivo production of bovine embryos from superovulated donors  
10.1530/REP-08-0362. *Reproduction* 2009; 137:161-180
5. Sessions-Bresnahan DR, Carnevale EM. The effect of equine metabolic syndrome on the ovarian follicular environment<sup>1</sup>. *Journal of Animal Science* 2014; 92:1485-1494
6. Sessions DR, Reedy SE, Vick MM, Murphy BA, Fitzgerald BP. Development of a model for inducing transient insulin resistance in the mare: Preliminary implications regarding the estrous cycle. *J Anim Sci* 2004; 82:2321-2328

7. Wu S, Divall S, Nwaopara A, Radovick S, Wondisford F, Ko C, Wolfe A. Obesity-Induced Infertility and Hyperandrogenism Are Corrected by Deletion of the Insulin Receptor in the Ovarian Theca Cell. *Diabetes* 2014; 63:1270-1282
8. Donadeu F, Ginther O. Changes in concentrations of follicular fluid factors during follicle selection in mares. *Biology of reproduction* 2002; 66:1111-1118
9. Donadeu F, Ginther O. Follicular waves and circulating concentrations of gonadotrophins, inhibin and oestradiol during the anovulatory season in mares. *Reproduction* 2002; 124:875-885
10. Baerwald AR, Adams GP, Pierson RA. Characterization of ovarian follicular wave dynamics in women. *Biology of reproduction* 2003; 69:1023-1031
11. Ginther O, Beg M, Gastal M, Gastal E. Follicle dynamics and selection in mares. *Anim Reprod* 2004; 1:45-63
12. Ginther O. Major and minor follicular waves during the equine estrous cycle. *Journal of equine veterinary science* 1993; 13:18-25
13. Ginther O. Reproductive biology of the mare-basic and applied aspects. *Reproductive biology of the mare-basic and applied aspects* 1979;
14. Ginther O, Beg M, Gastal E, Gastal M, Baerwald A, Pierson R. Systemic concentrations of hormones during the development of follicular waves in mares and women: a comparative study. *Reproduction* 2005; 130:379-388
15. Ginther O, Gastal E, Gastal M, Checura C, Beg M. Dose-response study of intrafollicular injection of insulin-like growth factor-I on follicular fluid factors and follicle dominance in mares. *Biology of reproduction* 2004; 70:1063-1069

16. Zhou P, Baumgarten SC, Wu Y, Bennett J, Winston N, Hirshfeld-Cytron J, Stocco C. IGF-I signaling is essential for FSH stimulation of AKT and steroidogenic genes in granulosa cells. *Molecular endocrinology* 2013; 27:511-523
17. Erickson GF, Garzo VG, Magooffin DA. Insulin-like growth factor-I regulates aromatase activity in human granulosa and granulosa luteal cells. *The Journal of Clinical Endocrinology & Metabolism* 1989; 69:716-724
18. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nature reviews Molecular cell biology* 2001; 2:127
19. Park J-Y, Su Y-Q, Ariga M, Law E, Jin S-LC, Conti M. EGF-like growth factors as mediators of LH action in the ovulatory follicle. *Science* 2004; 303:682-684
20. Eppig JJ. Oocyte-somatic cell communication in the ovarian follicles of mammals. Paper presented at: *Seminars in Developmental Biology* 1994
21. Albertini DF, Combelles C, Benecchi E, Carabatsos MJ. Cellular basis for paracrine regulation of ovarian follicle development. *Reproduction* 2001; 121:647-653
22. Makabe S, Naguro T, Stallone T. Oocyte–follicle cell interactions during ovarian follicle development, as seen by high resolution scanning and transmission electron microscopy in humans. *Microscopy research and technique* 2006; 69:436-449
23. Te Velde ER, Pearson PL. The variability of female reproductive ageing. *Human reproduction update* 2002; 8:141-154
24. Sherman BM, West JH, Korenman SG. The menopausal transition: analysis of LH, FSH, estradiol, and progesterone concentrations during menstrual cycles of older women. *The Journal of Clinical Endocrinology & Metabolism* 1976; 42:629-636

25. Lenton E, Sexton L, Lee S, Cooke I. Progressive changes in LH and FSH and LH: FSH ratio in women throughout reproductive life. *Maturitas* 1988; 10:35-43
26. Healy DL, Trounson AO, Andersen AN. Female infertility: causes and treatment. *The Lancet* 1994; 343:1539-1544
27. Te Velde E, Scheffer G, Dorland M, Broekmans F, Fauser B. Developmental and endocrine aspects of normal ovarian aging. *Molecular and cellular endocrinology* 1998; 145:67-73
28. Madill S. Reproductive considerations: mare and stallion. *The Veterinary clinics of North America Equine practice* 2002; 18:591-619
29. Unger RH, Zhou Y-T, Orci L. Regulation of fatty acid homeostasis in cells: novel role of leptin. *Proceedings of the National Academy of Sciences* 1999; 96:2327-2332
30. Wang Z-w, Pan W-t, Lee Y, Kakuma T, Zhou Y-t, Unger RH. The role of leptin resistance in the lipid abnormalities of aging. *The FASEB Journal* 2001; 15:108-114
31. Heine P, Taylor J, Iwamoto G, Lubahn D, Cooke P. Increased adipose tissue in male and female estrogen receptor- $\alpha$  knockout mice. *Proceedings of the National Academy of Sciences* 2000; 97:12729-12734
32. Jones ME, Boon WC, Proietto J, Simpson ER. Of mice and men: the evolving phenotype of aromatase deficiency. *Trends in Endocrinology & Metabolism* 2006; 17:55-64
33. Wich BK, Carnes M. Menopause and the aging female reproductive system. *Endocrinology and metabolism clinics of North America* 1995; 24:273-295
34. Corton JM, Gillespie JG, Hardie DG. Role of the AMP-activated protein kinase in the cellular stress response. *Current biology* 1994; 4:315-324

35. Kahn BB, Alquier T, Carling D, Hardie DG. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. *Cell metabolism* 2005; 1:15-25
36. Hardie DG, Carling D, Carlson M. The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? : Annual Reviews 4139 El Camino Way, PO Box 10139, Palo Alto, CA 94303-0139, USA; 1998.
37. Kemp B, Stapleton D, Campbell D, Chen Z-P, Murthy S, Walter M, Gupta A, Adams J, Katsis F, van Denderen B. AMP-activated protein kinase, super metabolic regulator. Portland Press Limited; 2003.
38. Adams J, Chen ZP, Van Denderen BJ, Morton CJ, Parker MW, Witters LA, Stapleton D, Kemp BE. Intrasteric control of AMPK via the  $\gamma$ 1 subunit AMP allosteric regulatory site. *Protein science* 2004; 13:155-165
39. Scott JW, Hawley SA, Green KA, Anis M, Stewart G, Scullion GA, Norman DG, Hardie DG. CBS domains form energy-sensing modules whose binding of adenosine ligands is disrupted by disease mutations. *J Clin Invest* 2004; 113:274-284
40. Park SH, Gammon SR, Knippers JD, Paulsen SR, Rubink DS, Winder WW. Phosphorylation-activity relationships of AMPK and acetyl-CoA carboxylase in muscle. *Journal of Applied Physiology* 2002; 92:2475-2482
41. Chen Z-P, Stephens TJ, Murthy S, Canny BJ, Hargreaves M, Witters LA, Kemp BE, McConnell GK. Effect of Exercise Intensity on Skeletal Muscle AMPK Signaling in Humans. *Diabetes* 2003; 52:2205-2212

42. Barnes BR, Long YC, Steiler TL, Leng Y, Galuska D, Wojtaszewski JFP, Andersson L, Zierath JR. Changes in Exercise-Induced Gene Expression in 5'-AMP-Activated Protein Kinase  $\gamma$ 3-Null and  $\gamma$ 3 R225Q Transgenic Mice. *Diabetes* 2005; 54:3484-3489
43. Inoki K, Li Y, Zhu T, Wu J, Guan K-L. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nature cell biology* 2002; 4:648
44. Inoki K, Zhu T, Guan K-L. TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 2003; 115:577-590
45. Ning J, Clemmons DR. AMP-activated protein kinase inhibits IGF-I signaling and protein synthesis in vascular smooth muscle cells via stimulation of insulin receptor substrate 1 S794 and tuberous sclerosis 2 S1345 phosphorylation. *Molecular endocrinology* 2010; 24:1218-1229
46. Winder Wa, Hardie D. AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. *American Journal of Physiology-Endocrinology And Metabolism* 1999; 277:E1-E10
47. Raymond R, Russell I, Bergeron R, Shulman GI, Young LH. Translocation of myocardial GLUT-4 and increased glucose uptake through activation of AMPK by AICAR. *American Journal of Physiology-Heart and Circulatory Physiology* 1999; 277:H643-H649
48. Yang J, Holman GD. Insulin and Contraction Stimulate Exocytosis, but Increased AMP-activated Protein Kinase Activity Resulting from Oxidative Metabolism Stress Slows Endocytosis of GLUT4 in Cardiomyocytes. *Journal of Biological Chemistry* 2005; 280:4070-4078

49. Salminen A, Kaarniranta K. AMP-activated protein kinase (AMPK) controls the aging process via an integrated signaling network. *Ageing research reviews* 2012; 11:230-241
50. Hardie DG, Hawley SA. AMP-activated protein kinase: the energy charge hypothesis revisited. *BioEssays* 2001; 23:1112-1119
51. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, Shulman GI. Mitochondrial Dysfunction in the Elderly: Possible Role in Insulin Resistance. *Science* 2003; 300:1140-1142
52. Wang W, Yang X, López de Silanes I, Carling D, Gorospe M. Increased AMP:ATP Ratio and AMP-activated Protein Kinase Activity during Cellular Senescence Linked to Reduced HuR Function. *Journal of Biological Chemistry* 2003; 278:27016-27023
53. Qiang W, Weiqiang K, Qing Z, Pengju Z, Yi L. Aging impairs insulin-stimulated glucose uptake in rat skeletal muscle via suppressing AMPK $\alpha$ . *Experimental & Molecular Medicine* 2007; 39:535
54. Reznick RM, Zong H, Li J, Morino K, Moore IK, Yu HJ, Liu Z-X, Dong J, Mustard KJ, Hawley SA, Befroy D, Pypaert M, Hardie DG, Young LH, Shulman GI. Aging-Associated Reductions in AMP-Activated Protein Kinase Activity and Mitochondrial Biogenesis. *Cell Metabolism* 2007; 5:151-156
55. Mulligan JD, Gonzalez AA, Kumar R, Davis AJ, Saupe KW. Aging Elevates Basal Adenosine Monophosphate-Activated Protein Kinase (AMPK) Activity and Eliminates Hypoxic Activation of AMPK in Mouse Liver. *The Journals of Gerontology: Series A* 2005; 60:21-27
56. Mariotti S, Franceschi C, Cossarizza A, Pinchera A. The aging thyroid. *Endocrine reviews* 1995; 16:686-715

57. Hijmans W, Radil J, Bottazzo G, Doniach D. Autoantibodies in highly aged humans. *Mechanisms of ageing and development* 1984; 26:83-89
58. Mariotti S, Barbesino G, Caturegli P, Giacomelli T, Pinchera A, Monti D, Cossarizza A, Franceschi C, Sansoni P, Passeri G. Thyroid and other organ-specific autoantibodies in healthy centenarians. *The Lancet* 1992; 339:1506-1508
59. Poretsky L. The insulin-related ovarian regulatory system in health and disease. *Endocr Rev* 1999; 20:535-582
60. de Boer JAM, van der Meer M, van der Veen EA, Schoemaker J. Growth Hormone (GH) Substitution in Hypogonadotropic, GH-Deficient Women Decreases the Follicle-Stimulating Hormone Threshold for Monofollicular Growth. *The Journal of Clinical Endocrinology & Metabolism* 1999; 84:590-595
61. Freeman WM, Walker SJ, Vrana KE. Quantitative RT-PCR: pitfalls and potential. *Biotechniques* 1999; 26:112-125
62. Morrison TB, Weis JJ, Wittwer CT. Quantification of low-copy transcripts by continuous SYBR Green I monitoring during amplification. *Biotechniques* 1998; 24:954-958, 960, 962
63. Pfaffl M. Development and validation of an externally standardised quantitative insulin-like growth factor-1 RT-PCR using LightCycler SYBR Green I technology. *Rapid Cycle Real-Time PCR: Springer*; 2001:281-291.
64. Ferre F. Quantitative or semi-quantitative PCR: reality versus myth. *Genome Research* 1992; 2:1-9
65. Souza F, Ntodou-Thome A, Tran C, Rostene W, Forgez P. Quantitative RT-PCR: limits and accuracy. *Biotechniques* 1996; 21:280-285

66. Overbergh L, Valckx D, Waer M, Mathieu C. Quantification of murine cytokine mRNAs using real time quantitative reverse transcriptase PCR. *Cytokine* 1999; 11:305-312
67. Marten N, Burke E, Hayden J, Straus D. Effect of amino acid limitation on the expression of 19 genes in rat hepatoma cells. *The FASEB Journal* 1994; 8:538-544
68. Foss DL, Baarsch MJ, Murtaugh MP. Regulation of hypoxanthine phosphoribosyltransferase, glyceraldehyde-3-phosphate dehydrogenase and  $\beta$ -actin mRNA expression in porcine immune cells and tissues. *Animal biotechnology* 1998; 9:67-78
69. Thellin O, Zorzi W, Lakaye B, De Borman B, Coumans B, Hennen G, Grisar T, Igout A, Heinen E. Housekeeping genes as internal standards: use and limits. *Journal of biotechnology* 1999; 75:291-295
70. Bhatia P, Taylor WR, Greenberg AH, Wright JA. Comparison of glyceraldehyde-3-phosphate dehydrogenase and 28S-ribosomal RNA gene expression as RNA loading controls for northern blot analysis of cell lines of varying malignant potential. *Analytical biochemistry* 1994; 216:223-226
71. Bereta J, Bereta M. Stimulation of glyceraldehyde-3-phosphate dehydrogenase mRNA levels by endogenous nitric oxide in cytokine-activated endothelium. *Biochemical and biophysical research communications* 1995; 217:363-369
72. Zhang J, Snyder SH. Nitric oxide stimulates auto-ADP-ribosylation of glyceraldehyde-3-phosphate dehydrogenase. *Proceedings of the National Academy of Sciences* 1992; 89:9382-9385

73. Carnevale EM, Maclellan LJ, Coutinho da Silva MA, Scott TJ, Squires EL. Comparison of culture and insemination techniques for equine oocyte transfer. *Theriogenology* 2000; 54:981-987
74. Overbergh L, Giulietti A, Valckx D, Decallonne B, Bouillon R, Mathieu C. The Use of Real-Time Reverse Transcriptase PCR for the Quantification of Cytokine Gene Expression. *Journal of Biomolecular Techniques : JBT* 2003; 14:33-43
75. Klein C, Rutllant J, Troedsson MH. Expression stability of putative reference genes in equine endometrial, testicular, and conceptus tissues. *BMC Research Notes* 2011; 4:120
76. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative CT method. *Nature Protocols* 2008; 3:1101
77. Blanquart C, Gonzalez-Yanes C, Tssad T. Monitoring the activation state of insulin/insulin-like growth factor-1 hybrid receptors using bioluminescence resonance energy transfer. *Molecular Pharmacology* 2006; 70:1802-1811
78. Stapleton D, Woollatt E, Mitchelhill KI, Nicholl JK, Fernandez CS, Michell BJ, Witters LA, Power DA, Sutherland GR, Kemp BE. AMP-activated protein kinase isoenzyme family: subunit structure and chromosomal location. *FEBS Letters* 1997; 409:452-456
79. Fox CJ, Hammerman PS, Thompson CB. Fuel feeds function: energy metabolism and the T-cell response. *Nature Reviews Immunology* 2005; 5:844
80. Olshansky SJ, Passaro DJ, Hershov RC, Layden J, Carnes BA, Brody J, Hayflick L, Butler RN, Allison DB, Ludwig DS. A Potential Decline in Life Expectancy in the United States in the 21st Century. *New England Journal of Medicine* 2005; 352:1138-1145

- 81.** Sessions-Bresnahan D, Carnevale E. The effect of equine metabolic syndrome on the ovarian follicular environment 1. *Journal of animal science* 2014; 92:1485-1494
- 82.** Sessions-Bresnahan DR, Heuberger AL, Carnevale EM. Obesity in mares promotes uterine inflammation and alters embryo lipid fingerprints and homeostasis. *Biology of reproduction* 2018;
- 83.** Hoffman RM, Boston RC, Stefanovski D, Kronfeld DS, Harris PA. Obesity and diet affect glucose dynamics and insulin sensitivity in Thoroughbred geldings1. *Journal of Animal Science* 2003; 81:2333-2342
- 84.** Potter S, Bamford N, Harris P, Bailey S. Incidence of laminitis and survey of dietary and management practices in pleasure horses and ponies in south-eastern Australia. *Australian Veterinary Journal* 2017; 95:370-374
- 85.** USDA L, Lameness V. Laminitis in US Horses. USDA: APHIS: VS, CEAH, National Animal Health Monitoring System N 2000; 348
- 86.** Frank N. Endocrinopathic Laminitis, Obesity-Associated Laminitis, and Pasture-Associated Laminitis.
- 87.** Karikoski NP, Horn I, McGowan TW, McGowan CM. The prevalence of endocrinopathic laminitis among horses presented for laminitis at a first-opinion/referral equine hospital. *Domestic Animal Endocrinology* 2011; 41:111-117
- 88.** Asplin KE, Sillence MN, Pollitt CC, McGowan CM. Induction of laminitis by prolonged hyperinsulinaemia in clinically normal ponies. *The Veterinary Journal* 2007; 174:530-535
- 89.** de Laat MA, Patterson-Kane JC, Pollitt CC, Sillence MN, McGowan CM. Histological and morphometric lesions in the pre-clinical, developmental phase of insulin-induced laminitis in Standardbred horses. *The Veterinary Journal* 2013; 195:305-312

90. Rachon D, Vortherms T, Seidlovä-Wuttke D, Wuttke W. Effects of dietary equol on body weight gain, intra-abdominal fat accumulation, plasma lipids, and glucose tolerance in ovariectomized Sprague-Dawley rats. *Menopause* 2007; 14:925-932
91. Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS. Increased adipose tissue in male and female estrogen receptor- $\alpha$  knockout mice. *Proceedings of the National Academy of Sciences* 2000; 97:12729-12734
92. Jones MEE, Chin Boon W, Proietto J, Simpson ER. Of mice and men: the evolving phenotype of aromatase deficiency. *Trends in Endocrinology & Metabolism* 2006; 17:55-64
93. Houmard JA, Weidner MD, Dolan PL, Leggett-Frazier N, Gavigan KE, Hickey MS, Tyndall GL, Zheng D, Alshami A, Dohm GL. Skeletal Muscle GLUT4 Protein Concentration and Aging in Humans. *Diabetes* 1995; 44:555-560
94. Larkin LM, Reynolds TH, Supiano MA, Kahn BB, Halter JB. Effect of Aging and Obesity on Insulin Responsiveness and Glut-4 Glucose Transporter Content in Skeletal Muscle of Fischer 344  $\times$  Brown Norway Rats. *The Journals of Gerontology: Series A* 2001; 56:B486-B492
95. Diamanti-Kandarakis E, Chatzigeorgiou A, Papageorgiou E, Koundouras D, Koutsilieris M. Advanced glycation end-products and insulin signaling in granulosa cells. *Experimental Biology and Medicine* 2015; 241:1438-1445
96. Katsumata M, Kaji Y, Takada R, Dauncey M. Nutritional regulation of GLUT expression, glucose metabolism, and intramuscular fat content in porcine muscle. *Asian Australasian Journal of Animal Sciences* 2007; 20:1297

97. Hardie D. AMPK: a key regulator of energy balance in the single cell and the whole organism. *International journal of obesity* 2008; 32:S7
98. Steinberg GR, Kemp BE. AMPK in health and disease. *Physiological reviews* 2009; 89:1025-1078
99. Suwa M, Nakano H, Kumagai S. Effects of chronic AICAR treatment on fiber composition, enzyme activity, UCP3, and PGC-1 in rat muscles. *Journal of applied physiology* 2003; 95:960-968
100. Jäger S, Handschin C, Pierre JS-, Spiegelman BM. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1 $\alpha$ . *Proceedings of the National Academy of Sciences* 2007; 104:12017-12022
101. Foretz M, Carling D, Guichard C, Ferré P, Foufelle F. AMP-activated protein kinase inhibits the glucose-activated expression of fatty acid synthase gene in rat hepatocytes. *Journal of Biological Chemistry* 1998; 273:14767-14771
102. Woods A, Azzout-Marniche D, Foretz M, Stein SC, Lemarchand P, Ferré P, Foufelle F, Carling D. Characterization of the role of AMP-activated protein kinase in the regulation of glucose-activated gene expression using constitutively active and dominant negative forms of the kinase. *Molecular and cellular biology* 2000; 20:6704-6711
103. Sessions-Bresnahan DR, Carnevale EM. Age-associated changes in granulosa cell transcript abundance in equine preovulatory follicles. *Reproduction, Fertility and Development* 2015; 27:906-913
104. Matoba S, Bender K, Fahey AG, Mamo S, Brennan L, Lonergan P, Fair T. Predictive value of bovine follicular components as markers of oocyte developmental potential. *Reproduction, Fertility and Development* 2014; 26:337-345

- 105.** Vick MM, Adams AA, Murphy BA, Sessions DR, Horohov DW, Cook RF, Shelton BJ, Fitzgerald BP. Relationships among inflammatory cytokines, obesity, and insulin sensitivity in the horse  
10.2527/jas.2006-673. J Anim Sci 2007; 85:1144-1155
- 106.** Dupont J, Scaramuzzi RJ. Insulin signalling and glucose transport in the ovary and ovarian function during the ovarian cycle. Biochemical Journal 2016; 473:1483-1501
- 107.** Gerlier D, Thomasset N. Use of MTT colorimetric assay to measure cell activation. Journal of Immunological Methods 1986; 94:57-63
- 108.** Baranao JLS, Hammond JM. Comparative effects of insulin and insulin-like growth factors on DNA synthesis and differentiation of porcine granulosa cells. Biochemical and Biophysical Research Communications 1984; 124:484-490
- 109.** Glistler C, Tannetta DS, Groome NP, Knight PG. Interactions Between Follicle-Stimulating Hormone and Growth Factors in Modulating Secretion of Steroids and Inhibin-Related Peptides by Nonluteinized Bovine Granulosa Cells1. Biology of Reproduction 2001; 65:1020-1028
- 110.** Winter PW, Al-Qatati A, Wolf-Ringwall AL, Schoeberl S, Chatterjee PB, Barisas BG, Roess DA, Crans DC. The anti-diabetic bis(maltolato)oxovanadium(iv) decreases lipid order while increasing insulin receptor localization in membrane microdomains. Dalton Transactions 2012; 41:6419-6430
- 111.** Saffarian S, Li U, Elson E, Pike L. Oligomerization of the EGF receptor investigated by life cell fluorescence intensity distribution analysis. Biophysical Journal 2007; 93:1021-1031

- 112.** Wolf-Ringwall AL, Winter PW, Liu J, Van Orden AK, Roess DA, Barisas BG.  
Restricted lateral diffusion of luteinizing hormone receptors in membrane microdomains.  
Journal of Biological Chemistry 2011; 286:29818-29827

## LIST OF ABBREVIATIONS

ACTB	Beta-actin
AICAR	Aminoimidazole-4-carboxamide ribonucleotide
AKT	protein kinase B, also known as Akt
AMP	Activated protein kinase
AMPK	Adenosine monophosphate-activated protein kinase $\alpha$ : alpha subunit $\beta$ : beta subunit $\gamma$ : Gamma subunit
AR	Amphiregulin
ATP	Adenosine triphosphate
BMP-6	Bone morphogenetic protein-6
BMP-15	Bone morphogenetic protein-15
BTC	Beta cellulin
GVBD	Germinal vesicle breakdown
CaMKK $\beta$	Calamedulin-dependent protein kinase kinase
cAMP	Cyclic Activated protein kinase
CBS	Cystathione $\beta$ -Synthase
CBS1	Cystathione $\beta$ -Synthase repeats 1
CBS2	Cystathione $\beta$ -Synthase repeats 2
CBS3	Cystathione $\beta$ -Synthase repeats 3
CBS4	Cystathione $\beta$ -Synthase repeats 4
cDNA	Complementary DNA
CMB	Carbohydrate-binding molecule
CRTC – 1	Cyclic AMP-regulated transcriptional Co-activator-1
CTD	C-terminal Domine
DAF – 16	Abnormal dauer formation protein – 16
DHEA-S	Dehydroepiandrosterone - sulfate

DMEM	Dulbecco's modified minimum essential medium
DNA	Deoxynucleic acid
dsDNA	Double stranded DNA
e IGF-1	Equine Insulin like growth factor
E2	17 $\beta$ -estradiol
EDTA	Ethylenediamine tetraacetic Acid
EGF	Epidermal growth factor
EPI	Epiregulin
ErbBs	Epidermal growth factor receptor (EGFR) family, also known as ErbB receptors
FBS	Fetal Bovine serum
FCS	Fluorescence correlation spectroscopy
FoxO	Forkhead box protein O
FSH	Follicle stimulating hormone
GADPH	Glyceraldehyde-3 phosphate dehydrogenase
GDF-9	Growth and differentiation factor-9
GLUT4	Glucose transporter 4
GTP	Guanosine Triphosphate
hCG	Human chorionic gonadotropin
HKG	Housekeeping gene
IGF-1R	Insulin like growth factor receptor
IL-1	Interleukin-1
InsL3	Insulin-like peptide 3
IR	Insulin Receptor
IRS	Insulin Receptor substrate
IU	International Unit
IV	Intra Veins
LH	Luteinizing hormone
m	minus
m RNA	Messenger RNA
mTOR,	mammalian target of rapamycin
NF-KB	Nuclear factor – KB

NO	Nitric oxide
Nrf2	Nuclear factor-erythroid 2-related
NTD	N-terminal Domine
p	plus
P4	Progesterone
PBS	Phosphate buffer saline
PCH	Photon counting histogram
PCO:	Polycystic ovarian fibrosis
PGE2	Prostaglandin E2
PI	Post Injection
PI3K	phosphatidyl inositol 3-OH-kinase
PP	Protein phosphatase
PTEN	phosphatase and tensin homolog
qRT-PCR	quantitative Real-time reverse transcription polymerase chain reaction
Rab	Rab family of proteins is a member of the Ras superfamily of
RNA	monomeric Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
RT	Reverse transcription
SDHA	Succinate dehydrogenase complex
SIRT1	Silent information regular 1
SKN-1	Skinhead -1
T4	Thyroxine
TAK 1	Transforming growth factor- $\beta$ -activated kinase 1
TSC1/2	Tuberous sclerosis complex1/2
TSH	Thyrotropin Stimulating Hormone
UV	UltraViolet
VEGF	Vascular endothelial growth factor
$\Delta \Delta$ CT	Double Delta Ct
$\Delta$ CT	Delta cycle threshold

## APPENDIX I: $\Delta$ Ct RAW DATA

### KEY: GENES (T)

T1; AMPK $\alpha$ 1,  
T2; AMPK $\alpha$ 2,  
T3; AMPK $\beta$ 1,  
T4; AMPK $\beta$ 2,  
T5; AMPK $\gamma$ 1,  
T6; AMPK $\gamma$ 1,  
T7; AMPK $\gamma$ 3,  
T8; IGF-1,  
T9; GLUT4  
T10; IR

### KEY: Treatments (G)

G0: Control  
G1: Insulin  
G2: human chorionic gonadotropin hCG  
G3: EGF  
G4: IGF-1  
G5: Progesterone

### KEY: Media

m: minus (without FBS and glucose)  
p: plus (without FBS and glucose).

**TEST 1: ANOVA F-tests for each variable**

Gene	Effect	NumDF	DenDF	FValue	Rawp
T1	Age Cat	2	15	1.124087	0.350841
T1	Treatment	5	165	0.898507	0.483568
T1	Age Cat* Treatment	10	165	1.172924	0.312533
T1	Glucose	1	165	1.132597	0.288777
T1	Age Cat*Glucose	2	165	0.816783	0.443629
T1	Treatment *Glucose	5	165	0.940962	0.456049
T1	Age Cat* Treatment *Glucose	10	165	0.699855	0.723669
T10	Age Cat	2	15	1.001194	0.390715
T10	Treatment	5	165	1.879314	0.100562
T10	Age Cat* Treatment	10	165	0.305349	0.979013
T10	Glucose	1	165	8.648967	0.003743
T10	Age Cat*Glucose	2	165	0.229958	0.794821
T10	Treatment *Glucose	5	165	0.861692	0.508239
T10	Age Cat* Treatment *Glucose	10	165	0.966766	0.474407
T2	Age Cat	2	15	1.299408	0.301688
T2	Treatment	5	165	0.331476	0.893399
T2	Age Cat* Treatment	10	165	1.929902	0.044422
T2	Glucose	1	165	5.629937	0.018808
T2	Age Cat*Glucose	2	165	1.425449	0.243345
T2	Treatment *Glucose	5	165	1.200511	0.311193
T2	Age Cat* Treatment *Glucose	10	165	0.809315	0.61999
T3	Age Cat	2	15	3.599971	0.05285
T3	Treatment	5	165	1.316345	0.259524
T3	Age Cat* Treatment	10	165	1.200987	0.293807
T3	Glucose	1	165	1.271024	0.261212
T3	Age Cat*Glucose	2	165	3.323709	0.038446
T3	Treatment *Glucose	5	165	1.023454	0.405588
T3	Age Cat* Treatment *Glucose	10	165	1.261707	0.256122
T4	Age Cat	2	15	0.305942	0.740917
T4	Treatment	5	165	0.155681	0.978067
T4	Age Cat* Treatment	10	165	0.651057	0.76815
T4	Glucose	1	165	1.548906	0.215062
T4	Age Cat*Glucose	2	165	0.15869	0.853391
T4	Treatment *Glucose	5	165	0.221513	0.95287
T4	Age Cat* Treatment *Glucose	10	165	0.959542	0.480775
T5	Age Cat	2	15	0.434834	0.655278
T5	Treatment	5	165	0.694961	0.627959
T5	Age Cat* Treatment	10	165	1.322583	0.22216
T5	Glucose	1	165	0.2144	0.643951

Gene	Effect	NumDF	DenDF	FValue	Rawp
T5	Age Cat*Glucose	2	165	0.896129	0.410123
T5	Treatment *Glucose	5	165	0.560466	0.730182
T5	Age Cat* Treatment *Glucose	10	165	0.470051	0.907473
T6	Age Cat	2	15	0.265599	0.770278
T6	Treatment	5	165	1.483529	0.197825
T6	Age Cat* Treatment	10	165	0.735698	0.690065
T6	Glucose	1	165	0.943493	0.332803
T6	Age Cat*Glucose	2	165	0.703632	0.496264
T6	Treatment *Glucose	5	165	0.202632	0.961002
T6	Age Cat* Treatment *Glucose	10	165	0.839367	0.591419
T7	Age Cat	2	15	2.086496	0.158676
T7	Treatment	5	165	1.804357	0.114655
T7	Age Cat* Treatment t	10	165	0.79511	0.633539
T7	Glucose	1	165	0.046047	0.830355
T7	Age Cat*Glucose	2	165	0.215193	0.806612
T7	Treatment *Glucose	5	165	0.644138	0.66634
T7	Age Cat*Trt*Glucose	10	165	0.867583	0.564808
T8	Age Cat	2	15	0.2476	0.783801
T8	Treatment	5	165	0.559481	0.730933
T8	Age Cat* Treatment	10	165	1.057547	0.398096
T8	Glucose	1	165	0.579256	0.44769
T8	Age Cat*Glucose	2	165	0.886828	0.413915
T8	Treatment *Glucose	5	165	0.689378	0.632146
T8	Age Cat* Treatment *Glucose	10	165	0.907424	0.527798
T9	Age Cat	2	15	0.500882	0.61578
T9	Treatment	5	165	0.527274	0.755401
T9	Age Cat* Treatment	10	165	1.269034	0.251834
T9	Glucose	1	165	3.738331	0.054889
T9	Age Cat*Glucose	2	165	3.474421	0.033265
T9	Treatment *Glucose	5	165	0.468675	0.7992
T9	Age Cat* Treatment *Glucose	10	165	1.076989	0.382736

SUMSTATS TEST: Values needed to plot mean and standard error

Gene	Glucose	AgeCat	Treatment	n	mean	sd	SE
T1	m	Middle	G0	4	8.6231	1.3721	0.6861
T1	m	Middle	G1	4	8.6194	1.0755	0.5377
T1	m	Middle	G2	4	8.4269	0.3734	0.1867
T1	m	Middle	G3	4	8.685	1.9941	0.997
T1	m	Middle	G4	4	9.3338	1.625	0.8125
T1	m	Middle	G5	4	8.1244	0.9081	0.454
T1	m	Old	G0	8	6.835	1.2735	0.4502
T1	m	Old	G1	8	7.1216	1.1378	0.4023
T1	m	Old	G2	8	7.3397	1.4748	0.5214
T1	m	Old	G3	8	7.5547	1.4794	0.5231
T1	m	Old	G4	8	8.1544	1.8116	0.6405
T1	m	Old	G5	8	7.0178	1.844	0.652
T1	m	Young	G0	6	8.9479	3.0279	1.2362
T1	m	Young	G1	6	8.86	3.0948	1.2635
T1	m	Young	G2	6	8.5446	2.629	1.0733
T1	m	Young	G3	6	9.2467	2.7191	1.11
T1	m	Young	G4	6	8.1104	2.4204	0.9881
T1	m	Young	G5	6	8.7279	2.3787	0.9711
T1	p	Middle	G0	4	8.4125	1.9201	0.96
T1	p	Middle	G1	4	7.3046	1.4128	0.7064
T1	p	Middle	G2	4	8.8969	2.4963	1.2481
T1	p	Middle	G3	4	9.7519	1.3363	0.6682
T1	p	Middle	G4	4	8.221	1.2115	0.6057
T1	p	Middle	G5	4	9.2669	0.8458	0.4229
T1	p	Old	G0	8	7.5347	1.4177	0.5012
T1	p	Old	G1	8	7.6006	1.422	0.5028
T1	p	Old	G2	8	7.8275	2.2908	0.8099
T1	p	Old	G3	8	7.6025	1.3047	0.4613
T1	p	Old	G4	8	8.5397	2.0902	0.739
T1	p	Old	G5	8	7.865	1.89	0.6682
T1	p	Young	G0	6	9.5271	3.6938	1.508
T1	p	Young	G1	6	8.2908	2.4002	0.9799
T1	p	Young	G2	6	8.9817	2.2149	0.9042
T1	p	Young	G3	6	8.6504	2.4404	0.9963
T1	p	Young	G4	6	8.5213	2.1774	0.8889
T1	p	Young	G5	6	8.9288	2.2441	0.9162
T10	m	Middle	G0	4	8.3417	0.8715	0.4357
T10	m	Middle	G1	4	9.1969	0.4942	0.2471
T10	m	Middle	G2	4	9.1594	0.5594	0.2797

Gene	Glucose	AgeCat	Treatment	n	mean	sd	SE
T10	m	Middle	G3	4	9.1975	1.2234	0.6117
T10	m	Middle	G4	4	10.216	1.6546	0.8273
T10	m	Middle	G5	4	8.8394	0.7353	0.3677
T10	m	Old	G0	8	9.0988	1.6869	0.5964
T10	m	Old	G1	8	8.1891	1.5196	0.5373
T10	m	Old	G2	8	10.11	3.3605	1.1881
T10	m	Old	G3	8	10.501	3.6567	1.2928
T10	m	Old	G4	8	10.218	3.437	1.2152
T10	m	Old	G5	8	10.082	3.492	1.2346
T10	m	Young	G0	6	10.848	2.8521	1.1644
T10	m	Young	G1	6	10.21	1.7285	0.7057
T10	m	Young	G2	6	10.845	1.6156	0.6596
T10	m	Young	G3	6	11.396	1.9474	0.795
T10	m	Young	G4	6	10.944	2.7919	1.1398
T10	m	Young	G5	6	10.686	2.2969	0.9377
T10	p	Middle	G0	4	10.16	1.4806	0.7403
T10	p	Middle	G1	4	9.2783	1.1789	0.5895
T10	p	Middle	G2	4	9.3844	1.4971	0.7486
T10	p	Middle	G3	4	10.627	2.2408	1.1204
T10	p	Middle	G4	4	8.8294	0.7791	0.3895
T10	p	Middle	G5	4	10.327	1.3321	0.6661
T10	p	Old	G0	8	11.065	3.7503	1.3259
T10	p	Old	G1	8	9.6863	1.7089	0.6042
T10	p	Old	G2	8	9.0238	5.0144	1.7729
T10	p	Old	G3	8	10.378	4.4093	1.5589
T10	p	Old	G4	8	11.326	3.4029	1.2031
T10	p	Old	G5	8	10.288	3.6097	1.2762
T10	p	Young	G0	6	11.403	1.6198	0.6613
T10	p	Young	G1	6	11.121	2.1503	0.8779
T10	p	Young	G2	6	11.508	2.266	0.9251
T10	p	Young	G3	6	12.075	2.0331	0.83
T10	p	Young	G4	6	12.061	1.5783	0.6444
T10	p	Young	G5	6	12.366	2.8955	1.1821
T2	m	Middle	G0	4	9.76	2.08	1.04
T2	m	Middle	G1	4	9.42	0.37	0.19
T2	m	Middle	G2	4	9.02	1.34	0.67
T2	m	Middle	G3	4	10.38	2.93	1.47
T2	m	Middle	G4	4	10.41	2.26	1.13
T2	m	Middle	G5	4	9.58	2.93	1.47
T2	m	Old	G0	8	7.76	1.47	0.52
T2	m	Old	G1	8	8.45	1.70	0.60
T2	m	Old	G2	8	8.22	1.11	0.39

Gene	Glucose	AgeCat	Treatment	n	mean	sd	SE
T2	m	Old	G3	8	8.27	1.30	0.46
T2	m	Old	G4	8	9.21	1.38	0.49
T2	m	Old	G5	8	7.90	1.28	0.45
T2	m	Young	G0	6	10.34	2.97	1.21
T2	m	Young	G1	6	10.37	2.61	1.06
T2	m	Young	G2	6	8.97	2.65	1.08
T2	m	Young	G3	6	9.91	2.25	0.92
T2	m	Young	G4	6	9.37	2.26	0.92
T2	m	Young	G5	6	9.36	2.24	0.91
T2	p	Middle	G0	4	9.26	1.58	0.79
T2	p	Middle	G1	4	8.55	1.68	0.84
T2	p	Middle	G2	4	10.78	3.25	1.62
T2	p	Middle	G3	4	11.02	2.02	1.01
T2	p	Middle	G4	4	9.32	2.65	1.33
T2	p	Middle	G5	4	10.45	1.86	0.93
T2	p	Old	G0	8	9.18	1.43	0.51
T2	p	Old	G1	8	9.81	1.76	0.62
T2	p	Old	G2	8	8.95	1.21	0.43
T2	p	Old	G3	8	8.46	1.09	0.39
T2	p	Old	G4	8	9.59	1.58	0.56
T2	p	Old	G5	8	9.05	2.03	0.72
T2	p	Young	G0	6	10.98	3.24	1.32
T2	p	Young	G1	6	9.76	1.58	0.64
T2	p	Young	G2	6	9.91	2.37	0.97
T2	p	Young	G3	6	9.92	2.13	0.87
T2	p	Young	G4	6	9.72	1.86	0.76
T2	p	Young	G5	6	10.19	2.00	0.82
T3	m	Middle	G0	4	10.498	1.6726	0.8363
T3	m	Middle	G1	4	9.6644	1.7046	0.8523
T3	m	Middle	G2	4	9.6906	0.6595	0.3297
T3	m	Middle	G3	4	9.9113	2.0264	1.0132
T3	m	Middle	G4	4	10.804	1.4744	0.7372
T3	m	Middle	G5	4	9.4556	0.8516	0.4258
T3	m	Old	G0	8	7.8963	2.0214	0.7147
T3	m	Old	G1	8	7.7728	2.2947	0.8113
T3	m	Old	G2	8	8.2697	1.9582	0.6923
T3	m	Old	G3	8	8.5541	1.8547	0.6557
T3	m	Old	G4	8	8.7738	1.9932	0.7047
T3	m	Old	G5	8	8.0903	2.1051	0.7443
T3	m	Young	G0	6	11.359	2.5303	1.033
T3	m	Young	G1	6	11.15	2.0247	0.8266
T3	m	Young	G2	6	10.081	2.334	0.9529

Gene	Glucose	AgeCat	Treatment	n	mean	sd	SE
T3	m	Young	G3	6	10.55	1.1403	0.4655
T3	m	Young	G4	6	10.614	1.0561	0.4312
T3	m	Young	G5	6	10.263	1.2363	0.5047
T3	p	Middle	G0	4	9.8338	2.1299	1.0649
T3	p	Middle	G1	4	8.3458	0.9454	0.4727
T3	p	Middle	G2	4	9.5594	2.3388	1.1694
T3	p	Middle	G3	4	11.712	2.1486	1.0743
T3	p	Middle	G4	4	9.4573	0.8326	0.4163
T3	p	Middle	G5	4	10.437	0.5331	0.2665
T3	p	Old	G0	8	9.0741	1.8977	0.671
T3	p	Old	G1	8	9.1844	1.2816	0.4531
T3	p	Old	G2	8	8.9963	2.1496	0.76
T3	p	Old	G3	8	8.39	1.8621	0.6583
T3	p	Old	G4	8	9.5378	2.3349	0.8255
T3	p	Old	G5	8	9.1219	2.1147	0.7477
T3	p	Young	G0	6	11.118	2.8366	1.158
T3	p	Young	G1	6	10.298	1.763	0.7197
T3	p	Young	G2	6	10.012	2.0812	0.8497
T3	p	Young	G3	6	10.448	1.5772	0.6439
T3	p	Young	G4	6	10.534	1.2145	0.4958
T3	p	Young	G5	6	11.091	1.5827	0.6461
T4	m	Middle	G0	4	8.0931	1.0411	0.5205
T4	m	Middle	G1	4	8.3931	0.9336	0.4668
T4	m	Middle	G2	4	7.8431	0.7298	0.3649
T4	m	Middle	G3	4	7.8188	1.124	0.562
T4	m	Middle	G4	4	9.1325	1.3838	0.6919
T4	m	Middle	G5	4	8.1356	0.9294	0.4647
T4	m	Old	G0	8	7.7019	1.8055	0.6383
T4	m	Old	G1	8	7.3997	2.1394	0.7564
T4	m	Old	G2	8	8.4434	2.0517	0.7254
T4	m	Old	G3	8	8.0028	1.7461	0.6173
T4	m	Old	G4	8	7.7294	1.9531	0.6905
T4	m	Old	G5	8	7.5359	1.8666	0.6599
T4	m	Young	G0	6	8.5046	1.2257	0.5004
T4	m	Young	G1	6	8.3108	1.2531	0.5116
T4	m	Young	G2	6	8.4771	1.1548	0.4714
T4	m	Young	G3	6	8.6408	0.6446	0.2632
T4	m	Young	G4	6	7.9679	0.9899	0.4041
T4	m	Young	G5	6	7.9654	0.9606	0.3922
T4	p	Middle	G0	4	8.0675	1.2882	0.6441
T4	p	Middle	G1	4	7.1983	1.0657	0.5329
T4	p	Middle	G2	4	8.5906	2.0402	1.0201

Gene	Glucose	AgeCat	Treatment	n	mean	sd	SE
T4	p	Middle	G3	4	9.0331	1.5966	0.7983
T4	p	Middle	G4	4	7.9798	0.732	0.366
T4	p	Middle	G5	4	9.4344	1.4888	0.7444
T4	p	Old	G0	8	7.9366	1.8706	0.6614
T4	p	Old	G1	8	8.7181	3.1425	1.111
T4	p	Old	G2	8	8.4081	1.3192	0.4664
T4	p	Old	G3	8	7.9306	1.5293	0.5407
T4	p	Old	G4	8	8.2891	1.985	0.7018
T4	p	Old	G5	8	7.8456	2.707	0.9571
T4	p	Young	G0	6	9.1521	2.2612	0.9231
T4	p	Young	G1	6	8.5558	1.6692	0.6814
T4	p	Young	G2	6	8.405	0.9024	0.3684
T4	p	Young	G3	6	8.4479	0.9042	0.3692
T4	p	Young	G4	6	8.3221	0.7795	0.3182
T4	p	Young	G5	6	8.1529	0.9464	0.3864
T5	m	Middle	G0	4	10.001	2.1125	1.0562
T5	m	Middle	G1	4	8.5181	1.1397	0.5698
T5	m	Middle	G2	4	8.1419	0.4384	0.2192
T5	m	Middle	G3	4	8.3588	0.5527	0.2763
T5	m	Middle	G4	4	8.7925	1.0081	0.504
T5	m	Middle	G5	4	8.5181	0.3261	0.163
T5	m	Old	G0	8	10.622	4.5599	1.6122
T5	m	Old	G1	8	9.3284	4.9472	1.7491
T5	m	Old	G2	8	9.5622	4.2836	1.5145
T5	m	Old	G3	8	9.9491	3.7813	1.3369
T5	m	Old	G4	8	10.089	3.2288	1.1416
T5	m	Old	G5	8	9.6172	2.977	1.0525
T5	m	Young	G0	6	8.0971	2.2185	0.9057
T5	m	Young	G1	6	8.36	3.9158	1.5986
T5	m	Young	G2	6	9.0421	4.7446	1.937
T5	m	Young	G3	6	9.0792	4.345	1.7738
T5	m	Young	G4	6	8.2879	3.9333	1.6058
T5	m	Young	G5	6	7.7721	4.4779	1.8281
T5	p	Middle	G0	4	8.9025	2.0765	1.0383
T5	p	Middle	G1	4	7.7771	0.2898	0.1449
T5	p	Middle	G2	4	7.6856	0.7904	0.3952
T5	p	Middle	G3	4	8.9406	0.7326	0.3663
T5	p	Middle	G4	4	7.7623	0.0598	0.0299
T5	p	Middle	G5	4	9.2581	1.126	0.563
T5	p	Old	G0	8	10.555	4.7139	1.6666
T5	p	Old	G1	8	10.528	4.6669	1.65
T5	p	Old	G2	8	9.3419	2.6798	0.9475

Gene	Glucose	AgeCat	Treatment	n	mean	sd	SE
T5	p	Old	G3	8	9.8069	3.1495	1.1135
T5	p	Old	G4	8	10.563	4.1247	1.4583
T5	p	Old	G5	8	10.127	3.1281	1.106
T5	p	Young	G0	6	8.0546	1.7969	0.7336
T5	p	Young	G1	6	8.4958	3.8242	1.5612
T5	p	Young	G2	6	9.1742	3.9212	1.6008
T5	p	Young	G3	6	8.8763	4.1883	1.7099
T5	p	Young	G4	6	9.3446	4.2763	1.7458
T5	p	Young	G5	6	8.6779	3.1528	1.2871
T6	m	Middle	G0	4	14.618	3.3743	1.6871
T6	m	Middle	G1	4	15.042	4.3349	2.1674
T6	m	Middle	G2	4	14.387	4.3871	2.1935
T6	m	Middle	G3	4	12.235	4.3057	2.1529
T6	m	Middle	G4	4	13.83	1.309	0.6545
T6	m	Middle	G5	4	12.094	2.8656	1.4328
T6	m	Old	G0	8	13.215	5.2312	1.8495
T6	m	Old	G1	8	12.707	5.0629	1.79
T6	m	Old	G2	8	12.301	3.4839	1.2317
T6	m	Old	G3	8	12.589	3.1707	1.121
T6	m	Old	G4	8	13.404	1.9897	0.7035
T6	m	Old	G5	8	12.348	2.6024	0.9201
T6	m	Young	G0	6	13.933	4.577	1.8686
T6	m	Young	G1	6	12.408	3.4669	1.4153
T6	m	Young	G2	6	11.599	4.7094	1.9226
T6	m	Young	G3	6	13.185	3.5515	1.4499
T6	m	Young	G4	6	11.798	4.3069	1.7583
T6	m	Young	G5	6	13.256	4.2928	1.7525
T6	p	Middle	G0	4	15.605	4.6301	2.315
T6	p	Middle	G1	4	13.551	5.0233	2.5117
T6	p	Middle	G2	4	14.802	4.808	2.404
T6	p	Middle	G3	4	14.559	2.6467	1.3233
T6	p	Middle	G4	4	12.829	3.029	1.5145
T6	p	Middle	G5	4	15.032	5.2463	2.6231
T6	p	Old	G0	8	14.324	3.8533	1.3624
T6	p	Old	G1	8	13.978	3.0796	1.0888
T6	p	Old	G2	8	12.976	2.1368	0.7555
T6	p	Old	G3	8	12.117	3.3447	1.1825
T6	p	Old	G4	8	13.38	2.7187	0.9612
T6	p	Old	G5	8	12.553	4.0554	1.4338
T6	p	Young	G0	6	12.941	3.8987	1.5916
T6	p	Young	G1	6	12.773	4.3533	1.7772
T6	p	Young	G2	6	11.35	4.0463	1.6519

Gene	Glucose	AgeCat	Treatment	n	mean	sd	SE
T6	p	Young	G3	6	12.612	4.2326	1.7279
T6	p	Young	G4	6	12.404	3.7152	1.5167
T6	p	Young	G5	6	12.8	4.0037	1.6345
T7	m	Middle	G0	4	14.009	2.1231	1.0616
T7	m	Middle	G1	4	13.767	2.9659	1.4829
T7	m	Middle	G2	4	13.089	3.6893	1.8446
T7	m	Middle	G3	4	13.113	3.9953	1.9977
T7	m	Middle	G4	4	13.985	3.111	1.5555
T7	m	Middle	G5	4	12.832	2.8802	1.4401
T7	m	Old	G0	8	11.309	2.2894	0.8094
T7	m	Old	G1	8	10.213	3.1775	1.1234
T7	m	Old	G2	8	11.273	1.8599	0.6576
T7	m	Old	G3	8	13.313	3.6294	1.2832
T7	m	Old	G4	8	12.319	2.1754	0.7691
T7	m	Old	G5	8	10.669	2.7947	0.9881
T7	m	Young	G0	6	14.336	2.156	0.8802
T7	m	Young	G1	6	12.278	1.825	0.745
T7	m	Young	G2	6	12.935	2.9013	1.1845
T7	m	Young	G3	6	13.818	2.2789	0.9304
T7	m	Young	G4	6	14.23	1.8522	0.7561
T7	m	Young	G5	6	12.476	2.5419	1.0377
T7	p	Middle	G0	4	13.875	3.1363	1.5681
T7	p	Middle	G1	4	12.711	3.2681	1.634
T7	p	Middle	G2	4	14.432	4.5631	2.2815
T7	p	Middle	G3	4	12.734	2.4616	1.2308
T7	p	Middle	G4	4	12.459	3.4356	1.7178
T7	p	Middle	G5	4	13.273	1.6104	0.8052
T7	p	Old	G0	8	12.274	2.9202	1.0324
T7	p	Old	G1	8	11.819	2.5718	0.9093
T7	p	Old	G2	8	10.028	1.768	0.6251
T7	p	Old	G3	8	12.001	1.8846	0.6663
T7	p	Old	G4	8	12.617	2.4218	0.8562
T7	p	Old	G5	8	11.401	3.6636	1.2953
T7	p	Young	G0	6	13.541	1.259	0.514
T7	p	Young	G1	6	13.743	1.6043	0.6549
T7	p	Young	G2	6	12.724	2.7516	1.1233
T7	p	Young	G3	6	14.059	1.8573	0.7583
T7	p	Young	G4	6	13.885	1.7975	0.7338
T7	p	Young	G5	6	13.446	2.5231	1.03
T8	m	Middle	G0	4	10.487	0.5921	0.296
T8	m	Middle	G1	4	11.008	1.8627	0.9313
T8	m	Middle	G2	4	9.5906	3.0298	1.5149

Gene	Glucose	AgeCat	Treatment	n	mean	sd	SE
T8	m	Middle	G3	4	9.6425	3.1221	1.561
T8	m	Middle	G4	4	11.869	2.2698	1.1349
T8	m	Middle	G5	4	11.226	3.0311	1.5156
T8	m	Old	G0	8	10.543	3.8521	1.3619
T8	m	Old	G1	8	10.29	5.2171	1.8445
T8	m	Old	G2	8	11.284	4.4824	1.5848
T8	m	Old	G3	8	11.718	4.9287	1.7425
T8	m	Old	G4	8	10.278	4.2799	1.5132
T8	m	Old	G5	8	10.858	3.79	1.34
T8	m	Young	G0	6	8.6654	2.3957	0.9781
T8	m	Young	G1	6	8.7558	3.5435	1.4466
T8	m	Young	G2	6	9.6146	3.9047	1.5941
T8	m	Young	G3	6	9.8525	3.6707	1.4986
T8	m	Young	G4	6	10.498	3.7266	1.5214
T8	m	Young	G5	6	8.6229	3.8242	1.5612
T8	p	Middle	G0	4	11.35	1.3149	0.6575
T8	p	Middle	G1	4	10.008	2.7716	1.3858
T8	p	Middle	G2	4	10.324	2.4736	1.2368
T8	p	Middle	G3	4	8.7219	1.8692	0.9346
T8	p	Middle	G4	4	10.442	2.2573	1.1287
T8	p	Middle	G5	4	11.237	3.4236	1.7118
T8	p	Old	G0	8	12.421	4.0621	1.4362
T8	p	Old	G1	8	11.398	4.278	1.5125
T8	p	Old	G2	8	9.9725	4.9151	1.7378
T8	p	Old	G3	8	9.6531	4.5341	1.603
T8	p	Old	G4	8	11.507	4.0536	1.4332
T8	p	Old	G5	8	11.326	4.2759	1.5118
T8	p	Young	G0	6	8.7821	2.8279	1.1545
T8	p	Young	G1	6	9.975	3.8288	1.5631
T8	p	Young	G2	6	10.925	3.6508	1.4905
T8	p	Young	G3	6	10.448	3.5175	1.436
T8	p	Young	G4	6	10.299	4.2124	1.7197
T8	p	Young	G5	6	10.245	3.65	1.4901
T9	m	Middle	G0	4	19.668	0.9402	0.4701
T9	m	Middle	G1	4	19.093	2.1498	1.0749
T9	m	Middle	G2	4	18.432	2.3289	1.1645
T9	m	Middle	G3	4	18.349	2.7876	1.3938
T9	m	Middle	G4	4	20.72	1.1379	0.569
T9	m	Middle	G5	4	17.749	2.3174	1.1587
T9	m	Old	G0	8	17.044	4.8862	1.7275
T9	m	Old	G1	8	16.837	4.5265	1.6004
T9	m	Old	G2	8	17.166	4.0135	1.419

Gene	Glucose	AgeCat	Treatment	n	mean	sd	SE
T9	m	Old	G3	8	17.332	3.9155	1.3843
T9	m	Old	G4	8	17.704	4.3239	1.5287
T9	m	Old	G5	8	17.272	4.0402	1.4284
T9	m	Young	G0	6	16.551	4.8276	1.9709
T9	m	Young	G1	6	17.03	4.6457	1.8966
T9	m	Young	G2	6	17.578	5.4092	2.2083
T9	m	Young	G3	6	17.944	5.615	2.2923
T9	m	Young	G4	6	16.652	4.4929	1.8342
T9	m	Young	G5	6	16.816	4.7764	1.9499
T9	p	Middle	G0	4	20.662	0.5713	0.2856
T9	p	Middle	G1	4	19.481	2.6958	1.3479
T9	p	Middle	G2	4	19.509	1.6653	0.8327
T9	p	Middle	G3	4	19.169	1.6362	0.8181
T9	p	Middle	G4	4	19.219	1.736	0.868
T9	p	Middle	G5	4	20.453	2.2498	1.1249
T9	p	Old	G0	8	17.818	3.6	1.2728
T9	p	Old	G1	8	16.873	3.8154	1.349
T9	p	Old	G2	8	15.161	4.7613	1.6834
T9	p	Old	G3	8	16.456	4.4122	1.5599
T9	p	Old	G4	8	17.342	4.2805	1.5134
T9	p	Old	G5	8	17.611	5.1135	1.8079
T9	p	Young	G0	6	16.504	4.9788	2.0326
T9	p	Young	G1	6	18.865	5.0122	2.0462
T9	p	Young	G2	6	18.796	5.3152	2.1699
T9	p	Young	G3	6	18.478	5.151	2.1029
T9	p	Young	G4	6	19.45	5.5009	2.2458
T9	p	Young	G5	6	17.838	4.7201	1.927

**TEST 2:** Pairwise comparisons of main effects (Age, Category) for each variable, gene, or medium or combinations between treatments:

gene	St No	Effect	Slice	Trt	Trt	Estima	Std Err	DF	t val	Raw p	Adju m	Adj p
T1	1	Trt*Glucose	Glucose m	G0	G1	-0.06	0.4412	165	-0.1	0.883	Tukey-K	1
T1	1	Trt*Glucose	Glucose m	G0	G2	0.032	0.4412	165	0.07	0.943	Tukey-K	1
T1	1	Trt*Glucose	Glucose m	G0	G3	-0.36	0.4412	165	-0.8	0.416	Tukey-K	0.964
T1	1	Trt*Glucose	Glucose m	G0	G4	-0.4	0.4412	165	-0.9	0.369	Tukey-K	0.946
T1	1	Trt*Glucose	Glucose m	G0	G5	0.179	0.4412	165	0.4	0.686	Tukey-K	0.999
T1	1	Trt*Glucose	Glucose m	G1	G2	0.097	0.4412	165	0.22	0.827	Tukey-K	1
T1	1	Trt*Glucose	Glucose m	G1	G3	-0.3	0.4412	165	-0.7	0.504	Tukey-K	0.985
T1	1	Trt*Glucose	Glucose m	G1	G4	-0.33	0.4412	165	-0.8	0.452	Tukey-K	0.975
T1	1	Trt*Glucose	Glucose m	G1	G5	0.244	0.4412	165	0.55	0.582	Tukey-K	0.994
T1	1	Trt*Glucose	Glucose m	G2	G3	-0.39	0.4412	165	-0.9	0.376	Tukey-K	0.949
T1	1	Trt*Glucose	Glucose m	G2	G4	-0.43	0.4412	165	-1	0.332	Tukey-K	0.926
T1	1	Trt*Glucose	Glucose m	G2	G5	0.147	0.4412	165	0.33	0.739	Tukey-K	0.999
T1	1	Trt*Glucose	Glucose m	G3	G4	-0.04	0.4412	165	-0.1	0.933	Tukey-K	1
T1	1	Trt*Glucose	Glucose m	G3	G5	0.539	0.4412	165	1.22	0.224	Tukey-K	0.826
T1	1	Trt*Glucose	Glucose m	G4	G5	0.576	0.4412	165	1.31	0.193	Tukey-K	0.782
T1	1	Trt*Glucose	Glucose p	G0	G1	0.759	0.4412	165	1.72	0.087	Tukey-K	0.52
T1	1	Trt*Glucose	Glucose p	G0	G2	-0.08	0.4412	165	-0.2	0.861	Tukey-K	1
T1	1	Trt*Glucose	Glucose p	G0	G3	-0.18	0.4412	165	-0.4	0.689	Tukey-K	0.999
T1	1	Trt*Glucose	Glucose p	G0	G4	0.064	0.4412	165	0.15	0.885	Tukey-K	1
T1	1	Trt*Glucose	Glucose p	G0	G5	-0.2	0.4412	165	-0.4	0.658	Tukey-K	0.998
T1	1	Trt*Glucose	Glucose p	G1	G2	-0.84	0.4412	165	-1.9	0.06	Tukey-K	0.408
T1	1	Trt*Glucose	Glucose p	G1	G3	-0.94	0.4412	165	-2.1	0.035	Tukey-K	0.281
T1	1	Trt*Glucose	Glucose p	G1	G4	-0.7	0.4412	165	-1.6	0.117	Tukey-K	0.616
T1	1	Trt*Glucose	Glucose p	G1	G5	-0.95	0.4412	165	-2.2	0.032	Tukey-K	0.26
T1	1	Trt*Glucose	Glucose p	G2	G3	-0.1	0.4412	165	-0.2	0.822	Tukey-K	1
T1	1	Trt*Glucose	Glucose p	G2	G4	0.141	0.4412	165	0.32	0.749	Tukey-K	1
T1	1	Trt*Glucose	Glucose p	G2	G5	-0.12	0.4412	165	-0.3	0.789	Tukey-K	1
T1	1	Trt*Glucose	Glucose p	G3	G4	0.241	0.4412	165	0.55	0.586	Tukey-K	0.994
T1	1	Trt*Glucose	Glucose p	G3	G5	-0.02	0.4412	165	-0	0.966	Tukey-K	1
T1	1	Trt*Glucose	Glucose p	G4	G5	-0.26	0.4412	165	-0.6	0.557	Tukey-K	0.992
T10	1	Trt*Glucose	Glucose m	G0	G1	0.231	0.5937	165	0.39	0.698	Tukey-K	0.999
T10	1	Trt*Glucose	Glucose m	G0	G2	-0.61	0.5937	165	-1	0.307	Tukey-K	0.909
T10	1	Trt*Glucose	Glucose m	G0	G3	-0.94	0.5937	165	-1.6	0.117	Tukey-K	0.616
T10	1	Trt*Glucose	Glucose m	G0	G4	-1.03	0.5937	165	-1.7	0.085	Tukey-K	0.511
T10	1	Trt*Glucose	Glucose m	G0	G5	-0.44	0.5937	165	-0.7	0.46	Tukey-K	0.977
T10	1	Trt*Glucose	Glucose m	G1	G2	-0.84	0.5937	165	-1.4	0.159	Tukey-K	0.719
T10	1	Trt*Glucose	Glucose m	G1	G3	-1.17	0.5937	165	-2	0.051	Tukey-K	0.367
T10	1	Trt*Glucose	Glucose m	G1	G4	-1.26	0.5937	165	-2.1	0.035	Tukey-K	0.28
T10	1	Trt*Glucose	Glucose m	G1	G5	-0.67	0.5937	165	-1.1	0.26	Tukey-K	0.869

gene	No	Effect	Slice	Trt	Trt	Estima	Std Err	DF	t val	Raw p	Adju m	Adj p
T10	1	Trt*Glucose	Glucose m	G2	G3	-0.33	0.5937	165	-0.6	0.583	Tukey-K	0.994
T10	1	Trt*Glucose	Glucose m	G2	G4	-0.42	0.5937	165	-0.7	0.479	Tukey-K	0.981
T10	1	Trt*Glucose	Glucose m	G2	G5	0.169	0.5937	165	0.28	0.776	Tukey-K	1
T10	1	Trt*Glucose	Glucose m	G3	G4	-0.09	0.5937	165	-0.2	0.874	Tukey-K	1
T10	1	Trt*Glucose	Glucose m	G3	G5	0.496	0.5937	165	0.83	0.405	Tukey-K	0.961
T10	1	Trt*Glucose	Glucose m	G4	G5	0.59	0.5937	165	0.99	0.322	Tukey-K	0.919
T10	1	Trt*Glucose	Glucose p	G0	G1	0.848	0.5937	165	1.43	0.155	Tukey-K	0.71
T10	1	Trt*Glucose	Glucose p	G0	G2	0.904	0.5937	165	1.52	0.13	Tukey-K	0.65
T10	1	Trt*Glucose	Glucose p	G0	G3	-0.15	0.5937	165	-0.3	0.8	Tukey-K	1
T10	1	Trt*Glucose	Glucose p	G0	G4	0.137	0.5937	165	0.23	0.817	Tukey-K	1
T10	1	Trt*Glucose	Glucose p	G0	G5	-0.12	0.5937	165	-0.2	0.843	Tukey-K	1
T10	1	Trt*Glucose	Glucose p	G1	G2	0.056	0.5937	165	0.1	0.924	Tukey-K	1
T10	1	Trt*Glucose	Glucose p	G1	G3	-1	0.5937	165	-1.7	0.095	Tukey-K	0.546
T10	1	Trt*Glucose	Glucose p	G1	G4	-0.71	0.5937	165	-1.2	0.233	Tukey-K	0.838
T10	1	Trt*Glucose	Glucose p	G1	G5	-0.97	0.5937	165	-1.6	0.106	Tukey-K	0.583
T10	1	Trt*Glucose	Glucose p	G2	G3	-1.05	0.5937	165	-1.8	0.077	Tukey-K	0.484
T10	1	Trt*Glucose	Glucose p	G2	G4	-0.77	0.5937	165	-1.3	0.198	Tukey-K	0.789
T10	1	Trt*Glucose	Glucose p	G2	G5	-1.02	0.5937	165	-1.7	0.087	Tukey-K	0.52
T10	1	Trt*Glucose	Glucose p	G3	G4	0.288	0.5937	165	0.49	0.628	Tukey-K	0.997
T10	1	Trt*Glucose	Glucose p	G3	G5	0.033	0.5937	165	0.06	0.955	Tukey-K	1
T10	1	Trt*Glucose	Glucose p	G4	G5	-0.25	0.5937	165	-0.4	0.668	Tukey-K	0.998
T2	1	Trt*Glucose	Glucose m	G0	G1	-0.13	0.4703	165	-0.3	0.786	Tukey-K	1
T2	1	Trt*Glucose	Glucose m	G0	G2	0.546	0.4703	165	1.16	0.248	Tukey-K	0.855
T2	1	Trt*Glucose	Glucose m	G0	G3	-0.23	0.4703	165	-0.5	0.622	Tukey-K	0.996
T2	1	Trt*Glucose	Glucose m	G0	G4	-0.38	0.4703	165	-0.8	0.421	Tukey-K	0.966
T2	1	Trt*Glucose	Glucose m	G0	G5	0.339	0.4703	165	0.72	0.472	Tukey-K	0.979
T2	1	Trt*Glucose	Glucose m	G1	G2	0.673	0.4703	165	1.43	0.154	Tukey-K	0.708
T2	1	Trt*Glucose	Glucose m	G1	G3	-0.1	0.4703	165	-0.2	0.824	Tukey-K	1
T2	1	Trt*Glucose	Glucose m	G1	G4	-0.25	0.4703	165	-0.5	0.594	Tukey-K	0.995
T2	1	Trt*Glucose	Glucose m	G1	G5	0.467	0.4703	165	0.99	0.322	Tukey-K	0.92
T2	1	Trt*Glucose	Glucose m	G2	G3	-0.78	0.4703	165	-1.7	0.1	Tukey-K	0.564
T2	1	Trt*Glucose	Glucose m	G2	G4	-0.92	0.4703	165	-2	0.051	Tukey-K	0.366
T2	1	Trt*Glucose	Glucose m	G2	G5	-0.21	0.4703	165	-0.4	0.661	Tukey-K	0.998
T2	1	Trt*Glucose	Glucose m	G3	G4	-0.15	0.4703	165	-0.3	0.755	Tukey-K	1
T2	1	Trt*Glucose	Glucose m	G3	G5	0.572	0.4703	165	1.22	0.226	Tukey-K	0.829
T2	1	Trt*Glucose	Glucose m	G4	G5	0.718	0.4703	165	1.53	0.129	Tukey-K	0.647
T2	1	Trt*Glucose	Glucose p	G0	G1	0.436	0.4703	165	0.93	0.356	Tukey-K	0.939
T2	1	Trt*Glucose	Glucose p	G0	G2	-0.07	0.4703	165	-0.2	0.88	Tukey-K	1
T2	1	Trt*Glucose	Glucose p	G0	G3	0.006	0.4703	165	0.01	0.99	Tukey-K	1
T2	1	Trt*Glucose	Glucose p	G0	G4	0.266	0.4703	165	0.57	0.572	Tukey-K	0.993
T2	1	Trt*Glucose	Glucose p	G0	G5	-0.09	0.4703	165	-0.2	0.85	Tukey-K	1
T2	1	Trt*Glucose	Glucose p	G1	G2	-0.51	0.4703	165	-1.1	0.283	Tukey-K	0.89

gene	t No	Effect	Slice	Trt	Trt	Estima	Std Err	DF	t val	Raw p	Adju m	Adj p
T2	1	Trt*Glucose	Glucose p	G1	G3	-0.43	0.4703	165	-0.9	0.362	Tukey-K	0.943
T2	1	Trt*Glucose	Glucose p	G1	G4	-0.17	0.4703	165	-0.4	0.719	Tukey-K	0.999
T2	1	Trt*Glucose	Glucose p	G1	G5	-0.52	0.4703	165	-1.1	0.266	Tukey-K	0.874
T2	1	Trt*Glucose	Glucose p	G2	G3	0.077	0.4703	165	0.16	0.87	Tukey-K	1
T2	1	Trt*Glucose	Glucose p	G2	G4	0.337	0.4703	165	0.72	0.474	Tukey-K	0.98
T2	1	Trt*Glucose	Glucose p	G2	G5	-0.02	0.4703	165	-0	0.969	Tukey-K	1
T2	1	Trt*Glucose	Glucose p	G3	G4	0.26	0.4703	165	0.55	0.581	Tukey-K	0.994
T2	1	Trt*Glucose	Glucose p	G3	G5	-0.1	0.4703	165	-0.2	0.84	Tukey-K	1
T2	1	Trt*Glucose	Glucose p	G4	G5	-0.36	0.4703	165	-0.8	0.451	Tukey-K	0.974
T3	1	Trt*Glucose	Glucose m	G0	G1	0.389	0.4529	165	0.86	0.392	Tukey-K	0.956
T3	1	Trt*Glucose	Glucose m	G0	G2	0.571	0.4529	165	1.26	0.21	Tukey-K	0.806
T3	1	Trt*Glucose	Glucose m	G0	G3	0.246	0.4529	165	0.54	0.588	Tukey-K	0.994
T3	1	Trt*Glucose	Glucose m	G0	G4	-0.15	0.4529	165	-0.3	0.748	Tukey-K	1
T3	1	Trt*Glucose	Glucose m	G0	G5	0.648	0.4529	165	1.43	0.154	Tukey-K	0.708
T3	1	Trt*Glucose	Glucose m	G1	G2	0.182	0.4529	165	0.4	0.689	Tukey-K	0.999
T3	1	Trt*Glucose	Glucose m	G1	G3	-0.14	0.4529	165	-0.3	0.753	Tukey-K	1
T3	1	Trt*Glucose	Glucose m	G1	G4	-0.53	0.4529	165	-1.2	0.239	Tukey-K	0.846
T3	1	Trt*Glucose	Glucose m	G1	G5	0.259	0.4529	165	0.57	0.568	Tukey-K	0.993
T3	1	Trt*Glucose	Glucose m	G2	G3	-0.32	0.4529	165	-0.7	0.475	Tukey-K	0.98
T3	1	Trt*Glucose	Glucose m	G2	G4	-0.72	0.4529	165	-1.6	0.116	Tukey-K	0.611
T3	1	Trt*Glucose	Glucose m	G2	G5	0.078	0.4529	165	0.17	0.864	Tukey-K	1
T3	1	Trt*Glucose	Glucose m	G3	G4	-0.39	0.4529	165	-0.9	0.388	Tukey-K	0.954
T3	1	Trt*Glucose	Glucose m	G3	G5	0.402	0.4529	165	0.89	0.376	Tukey-K	0.949
T3	1	Trt*Glucose	Glucose m	G4	G5	0.794	0.4529	165	1.75	0.081	Tukey-K	0.499
T3	1	Trt*Glucose	Glucose p	G0	G1	0.733	0.4529	165	1.62	0.108	Tukey-K	0.588
T3	1	Trt*Glucose	Glucose p	G0	G2	0.486	0.4529	165	1.07	0.285	Tukey-K	0.891
T3	1	Trt*Glucose	Glucose p	G0	G3	-0.17	0.4529	165	-0.4	0.7	Tukey-K	0.999
T3	1	Trt*Glucose	Glucose p	G0	G4	0.166	0.4529	165	0.37	0.715	Tukey-K	0.999
T3	1	Trt*Glucose	Glucose p	G0	G5	-0.21	0.4529	165	-0.5	0.647	Tukey-K	0.997
T3	1	Trt*Glucose	Glucose p	G1	G2	-0.25	0.4529	165	-0.5	0.587	Tukey-K	0.994
T3	1	Trt*Glucose	Glucose p	G1	G3	-0.91	0.4529	165	-2	0.047	Tukey-K	0.345
T3	1	Trt*Glucose	Glucose p	G1	G4	-0.57	0.4529	165	-1.3	0.212	Tukey-K	0.81
T3	1	Trt*Glucose	Glucose p	G1	G5	-0.94	0.4529	165	-2.1	0.039	Tukey-K	0.304
T3	1	Trt*Glucose	Glucose p	G2	G3	-0.66	0.4529	165	-1.5	0.146	Tukey-K	0.691
T3	1	Trt*Glucose	Glucose p	G2	G4	-0.32	0.4529	165	-0.7	0.48	Tukey-K	0.981
T3	1	Trt*Glucose	Glucose p	G2	G5	-0.69	0.4529	165	-1.5	0.127	Tukey-K	0.644
T3	1	Trt*Glucose	Glucose p	G3	G4	0.34	0.4529	165	0.75	0.453	Tukey-K	0.975
T3	1	Trt*Glucose	Glucose p	G3	G5	-0.03	0.4529	165	-0.1	0.941	Tukey-K	1
T3	1	Trt*Glucose	Glucose p	G4	G5	-0.37	0.4529	165	-0.8	0.41	Tukey-K	0.963
T4	1	Trt*Glucose	Glucose m	G0	G1	0.065	0.478	165	0.14	0.891	Tukey-K	1
T4	1	Trt*Glucose	Glucose m	G0	G2	-0.15	0.478	165	-0.3	0.747	Tukey-K	1
T4	1	Trt*Glucose	Glucose m	G0	G3	-0.05	0.478	165	-0.1	0.91	Tukey-K	1

gene	t No	Effect	Slice	Trt	Trt	Estima	Std Err	DF	t val	Raw p	Adju m	Adj p
T4	1	Trt*Glucose	Glucose m	G0	G4	-0.18	0.478	165	-0.4	0.712	Tukey-K	0.999
T4	1	Trt*Glucose	Glucose m	G0	G5	0.221	0.478	165	0.46	0.645	Tukey-K	0.997
T4	1	Trt*Glucose	Glucose m	G1	G2	-0.22	0.478	165	-0.5	0.646	Tukey-K	0.997
T4	1	Trt*Glucose	Glucose m	G1	G3	-0.12	0.478	165	-0.3	0.803	Tukey-K	1
T4	1	Trt*Glucose	Glucose m	G1	G4	-0.24	0.478	165	-0.5	0.613	Tukey-K	0.996
T4	1	Trt*Glucose	Glucose m	G1	G5	0.156	0.478	165	0.33	0.745	Tukey-K	1
T4	1	Trt*Glucose	Glucose m	G2	G3	0.1	0.478	165	0.21	0.834	Tukey-K	1
T4	1	Trt*Glucose	Glucose m	G2	G4	-0.02	0.478	165	-0	0.963	Tukey-K	1
T4	1	Trt*Glucose	Glucose m	G2	G5	0.376	0.478	165	0.79	0.433	Tukey-K	0.97
T4	1	Trt*Glucose	Glucose m	G3	G4	-0.12	0.478	165	-0.3	0.798	Tukey-K	1
T4	1	Trt*Glucose	Glucose m	G3	G5	0.275	0.478	165	0.58	0.566	Tukey-K	0.992
T4	1	Trt*Glucose	Glucose m	G4	G5	0.398	0.478	165	0.83	0.407	Tukey-K	0.961
T4	1	Trt*Glucose	Glucose p	G0	G1	0.228	0.478	165	0.48	0.634	Tukey-K	0.997
T4	1	Trt*Glucose	Glucose p	G0	G2	-0.08	0.478	165	-0.2	0.863	Tukey-K	1
T4	1	Trt*Glucose	Glucose p	G0	G3	-0.09	0.478	165	-0.2	0.859	Tukey-K	1
T4	1	Trt*Glucose	Glucose p	G0	G4	0.188	0.478	165	0.39	0.694	Tukey-K	0.999
T4	1	Trt*Glucose	Glucose p	G0	G5	-0.09	0.478	165	-0.2	0.847	Tukey-K	1
T4	1	Trt*Glucose	Glucose p	G1	G2	-0.31	0.478	165	-0.6	0.517	Tukey-K	0.987
T4	1	Trt*Glucose	Glucose p	G1	G3	-0.31	0.478	165	-0.7	0.513	Tukey-K	0.986
T4	1	Trt*Glucose	Glucose p	G1	G4	-0.04	0.478	165	-0.1	0.934	Tukey-K	1
T4	1	Trt*Glucose	Glucose p	G1	G5	-0.32	0.478	165	-0.7	0.504	Tukey-K	0.985
T4	1	Trt*Glucose	Glucose p	G2	G3	-0	0.478	165	-0	0.996	Tukey-K	1
T4	1	Trt*Glucose	Glucose p	G2	G4	0.271	0.478	165	0.57	0.572	Tukey-K	0.993
T4	1	Trt*Glucose	Glucose p	G2	G5	-0.01	0.478	165	-0	0.984	Tukey-K	1
T4	1	Trt*Glucose	Glucose p	G3	G4	0.274	0.478	165	0.57	0.568	Tukey-K	0.993
T4	1	Trt*Glucose	Glucose p	G3	G5	-0.01	0.478	165	-0	0.988	Tukey-K	1
T4	1	Trt*Glucose	Glucose p	G4	G5	-0.28	0.478	165	-0.6	0.558	Tukey-K	0.992
T5	1	Trt*Glucose	Glucose m	G0	G1	0.838	0.5099	165	1.64	0.102	Tukey-K	0.572
T5	1	Trt*Glucose	Glucose m	G0	G2	0.658	0.5099	165	1.29	0.199	Tukey-K	0.79
T5	1	Trt*Glucose	Glucose m	G0	G3	0.444	0.5099	165	0.87	0.385	Tukey-K	0.953
T5	1	Trt*Glucose	Glucose m	G0	G4	0.517	0.5099	165	1.01	0.312	Tukey-K	0.913
T5	1	Trt*Glucose	Glucose m	G0	G5	0.937	0.5099	165	1.84	0.068	Tukey-K	0.444
T5	1	Trt*Glucose	Glucose m	G1	G2	-0.18	0.5099	165	-0.4	0.725	Tukey-K	0.999
T5	1	Trt*Glucose	Glucose m	G1	G3	-0.39	0.5099	165	-0.8	0.441	Tukey-K	0.972
T5	1	Trt*Glucose	Glucose m	G1	G4	-0.32	0.5099	165	-0.6	0.53	Tukey-K	0.989
T5	1	Trt*Glucose	Glucose m	G1	G5	0.1	0.5099	165	0.2	0.845	Tukey-K	1
T5	1	Trt*Glucose	Glucose m	G2	G3	-0.21	0.5099	165	-0.4	0.676	Tukey-K	0.998
T5	1	Trt*Glucose	Glucose m	G2	G4	-0.14	0.5099	165	-0.3	0.782	Tukey-K	1
T5	1	Trt*Glucose	Glucose m	G2	G5	0.28	0.5099	165	0.55	0.584	Tukey-K	0.994
T5	1	Trt*Glucose	Glucose m	G3	G4	0.072	0.5099	165	0.14	0.887	Tukey-K	1
T5	1	Trt*Glucose	Glucose m	G3	G5	0.493	0.5099	165	0.97	0.335	Tukey-K	0.928
T5	1	Trt*Glucose	Glucose m	G4	G5	0.421	0.5099	165	0.83	0.41	Tukey-K	0.962

gene	t No	Effect	Slice	Trt	Trt	Estima	Std Err	DF	t val	Raw p	Adju m	Adj p
T5	1	Trt*Glucose	Glucose p	G0	G1	0.237	0.5099	165	0.47	0.642	Tukey-K	0.997
T5	1	Trt*Glucose	Glucose p	G0	G2	0.437	0.5099	165	0.86	0.393	Tukey-K	0.956
T5	1	Trt*Glucose	Glucose p	G0	G3	-0.04	0.5099	165	-0.1	0.942	Tukey-K	1
T5	1	Trt*Glucose	Glucose p	G0	G4	-0.05	0.5099	165	-0.1	0.918	Tukey-K	1
T5	1	Trt*Glucose	Glucose p	G0	G5	-0.18	0.5099	165	-0.4	0.719	Tukey-K	0.999
T5	1	Trt*Glucose	Glucose p	G1	G2	0.2	0.5099	165	0.39	0.696	Tukey-K	0.999
T5	1	Trt*Glucose	Glucose p	G1	G3	-0.27	0.5099	165	-0.5	0.591	Tukey-K	0.995
T5	1	Trt*Glucose	Glucose p	G1	G4	-0.29	0.5099	165	-0.6	0.57	Tukey-K	0.993
T5	1	Trt*Glucose	Glucose p	G1	G5	-0.42	0.5099	165	-0.8	0.41	Tukey-K	0.962
T5	1	Trt*Glucose	Glucose p	G2	G3	-0.47	0.5099	165	-0.9	0.354	Tukey-K	0.938
T5	1	Trt*Glucose	Glucose p	G2	G4	-0.49	0.5099	165	-1	0.338	Tukey-K	0.93
T5	1	Trt*Glucose	Glucose p	G2	G5	-0.62	0.5099	165	-1.2	0.225	Tukey-K	0.828
T5	1	Trt*Glucose	Glucose p	G3	G4	-0.02	0.5099	165	-0	0.976	Tukey-K	1
T5	1	Trt*Glucose	Glucose p	G3	G5	-0.15	0.5099	165	-0.3	0.774	Tukey-K	1
T5	1	Trt*Glucose	Glucose p	G4	G5	-0.13	0.5099	165	-0.3	0.798	Tukey-K	1
T6	1	Trt*Glucose	Glucose m	G0	G1	0.536	0.7894	165	0.68	0.498	Tukey-K	0.984
T6	1	Trt*Glucose	Glucose m	G0	G2	1.16	0.7894	165	1.47	0.144	Tukey-K	0.684
T6	1	Trt*Glucose	Glucose m	G0	G3	1.252	0.7894	165	1.59	0.115	Tukey-K	0.609
T6	1	Trt*Glucose	Glucose m	G0	G4	0.911	0.7894	165	1.15	0.25	Tukey-K	0.858
T6	1	Trt*Glucose	Glucose m	G0	G5	1.356	0.7894	165	1.72	0.088	Tukey-K	0.522
T6	1	Trt*Glucose	Glucose m	G1	G2	0.624	0.7894	165	0.79	0.431	Tukey-K	0.969
T6	1	Trt*Glucose	Glucose m	G1	G3	0.716	0.7894	165	0.91	0.366	Tukey-K	0.944
T6	1	Trt*Glucose	Glucose m	G1	G4	0.375	0.7894	165	0.48	0.635	Tukey-K	0.997
T6	1	Trt*Glucose	Glucose m	G1	G5	0.819	0.7894	165	1.04	0.301	Tukey-K	0.904
T6	1	Trt*Glucose	Glucose m	G2	G3	0.093	0.7894	165	0.12	0.907	Tukey-K	1
T6	1	Trt*Glucose	Glucose m	G2	G4	-0.25	0.7894	165	-0.3	0.753	Tukey-K	1
T6	1	Trt*Glucose	Glucose m	G2	G5	0.196	0.7894	165	0.25	0.804	Tukey-K	1
T6	1	Trt*Glucose	Glucose m	G3	G4	-0.34	0.7894	165	-0.4	0.666	Tukey-K	0.998
T6	1	Trt*Glucose	Glucose m	G3	G5	0.103	0.7894	165	0.13	0.896	Tukey-K	1
T6	1	Trt*Glucose	Glucose m	G4	G5	0.444	0.7894	165	0.56	0.574	Tukey-K	0.993
T6	1	Trt*Glucose	Glucose p	G0	G1	0.856	0.7894	165	1.08	0.28	Tukey-K	0.887
T6	1	Trt*Glucose	Glucose p	G0	G2	1.248	0.7894	165	1.58	0.116	Tukey-K	0.613
T6	1	Trt*Glucose	Glucose p	G0	G3	1.194	0.7894	165	1.51	0.132	Tukey-K	0.657
T6	1	Trt*Glucose	Glucose p	G0	G4	1.419	0.7894	165	1.8	0.074	Tukey-K	0.47
T6	1	Trt*Glucose	Glucose p	G0	G5	0.829	0.7894	165	1.05	0.295	Tukey-K	0.9
T6	1	Trt*Glucose	Glucose p	G1	G2	0.392	0.7894	165	0.5	0.621	Tukey-K	0.996
T6	1	Trt*Glucose	Glucose p	G1	G3	0.338	0.7894	165	0.43	0.669	Tukey-K	0.998
T6	1	Trt*Glucose	Glucose p	G1	G4	0.563	0.7894	165	0.71	0.476	Tukey-K	0.98
T6	1	Trt*Glucose	Glucose p	G1	G5	-0.03	0.7894	165	-0	0.972	Tukey-K	1
T6	1	Trt*Glucose	Glucose p	G2	G3	-0.05	0.7894	165	-0.1	0.946	Tukey-K	1
T6	1	Trt*Glucose	Glucose p	G2	G4	0.172	0.7894	165	0.22	0.828	Tukey-K	1
T6	1	Trt*Glucose	Glucose p	G2	G5	-0.42	0.7894	165	-0.5	0.596	Tukey-K	0.995

gene	t No	Effect	Slice	Trt	Trt	Estima	Std Err	DF	t val	Raw p	Adju m	Adj p
T6	1	Trt*Glucose	Glucose p	G3	G4	0.225	0.7894	165	0.29	0.776	Tukey-K	1
T6	1	Trt*Glucose	Glucose p	G3	G5	-0.37	0.7894	165	-0.5	0.644	Tukey-K	0.997
T6	1	Trt*Glucose	Glucose p	G4	G5	-0.59	0.7894	165	-0.7	0.455	Tukey-K	0.975
T7	1	Trt*Glucose	Glucose m	G0	G1	1.132	0.671	165	1.69	0.093	Tukey-K	0.542
T7	1	Trt*Glucose	Glucose m	G0	G2	0.786	0.671	165	1.17	0.243	Tukey-K	0.85
T7	1	Trt*Glucose	Glucose m	G0	G3	-0.2	0.671	165	-0.3	0.77	Tukey-K	1
T7	1	Trt*Glucose	Glucose m	G0	G4	-0.29	0.671	165	-0.4	0.663	Tukey-K	0.998
T7	1	Trt*Glucose	Glucose m	G0	G5	1.226	0.671	165	1.83	0.07	Tukey-K	0.451
T7	1	Trt*Glucose	Glucose m	G1	G2	-0.35	0.671	165	-0.5	0.606	Tukey-K	0.995
T7	1	Trt*Glucose	Glucose m	G1	G3	-1.33	0.671	165	-2	0.049	Tukey-K	0.359
T7	1	Trt*Glucose	Glucose m	G1	G4	-1.42	0.671	165	-2.1	0.035	Tukey-K	0.28
T7	1	Trt*Glucose	Glucose m	G1	G5	0.094	0.671	165	0.14	0.889	Tukey-K	1
T7	1	Trt*Glucose	Glucose m	G2	G3	-0.98	0.671	165	-1.5	0.145	Tukey-K	0.688
T7	1	Trt*Glucose	Glucose m	G2	G4	-1.08	0.671	165	-1.6	0.11	Tukey-K	0.595
T7	1	Trt*Glucose	Glucose m	G2	G5	0.44	0.671	165	0.66	0.513	Tukey-K	0.986
T7	1	Trt*Glucose	Glucose m	G3	G4	-0.1	0.671	165	-0.1	0.886	Tukey-K	1
T7	1	Trt*Glucose	Glucose m	G3	G5	1.422	0.671	165	2.12	0.036	Tukey-K	0.283
T7	1	Trt*Glucose	Glucose m	G4	G5	1.519	0.671	165	2.26	0.025	Tukey-K	0.215
T7	1	Trt*Glucose	Glucose p	G0	G1	0.472	0.671	165	0.7	0.483	Tukey-K	0.981
T7	1	Trt*Glucose	Glucose p	G0	G2	0.835	0.671	165	1.24	0.215	Tukey-K	0.814
T7	1	Trt*Glucose	Glucose p	G0	G3	0.299	0.671	165	0.45	0.657	Tukey-K	0.998
T7	1	Trt*Glucose	Glucose p	G0	G4	0.243	0.671	165	0.36	0.718	Tukey-K	0.999
T7	1	Trt*Glucose	Glucose p	G0	G5	0.523	0.671	165	0.78	0.436	Tukey-K	0.971
T7	1	Trt*Glucose	Glucose p	G1	G2	0.363	0.671	165	0.54	0.589	Tukey-K	0.994
T7	1	Trt*Glucose	Glucose p	G1	G3	-0.17	0.671	165	-0.3	0.796	Tukey-K	1
T7	1	Trt*Glucose	Glucose p	G1	G4	-0.23	0.671	165	-0.3	0.733	Tukey-K	0.999
T7	1	Trt*Glucose	Glucose p	G1	G5	0.051	0.671	165	0.08	0.939	Tukey-K	1
T7	1	Trt*Glucose	Glucose p	G2	G3	-0.54	0.671	165	-0.8	0.425	Tukey-K	0.967
T7	1	Trt*Glucose	Glucose p	G2	G4	-0.59	0.671	165	-0.9	0.379	Tukey-K	0.95
T7	1	Trt*Glucose	Glucose p	G2	G5	-0.31	0.671	165	-0.5	0.643	Tukey-K	0.997
T7	1	Trt*Glucose	Glucose p	G3	G4	-0.06	0.671	165	-0.1	0.934	Tukey-K	1
T7	1	Trt*Glucose	Glucose p	G3	G5	0.225	0.671	165	0.33	0.738	Tukey-K	0.999
T7	1	Trt*Glucose	Glucose p	G4	G5	0.28	0.671	165	0.42	0.677	Tukey-K	0.998
T8	1	Trt*Glucose	Glucose m	G0	G1	-0.12	0.7567	165	-0.2	0.875	Tukey-K	1
T8	1	Trt*Glucose	Glucose m	G0	G2	-0.26	0.7567	165	-0.3	0.727	Tukey-K	0.999
T8	1	Trt*Glucose	Glucose m	G0	G3	-0.51	0.7567	165	-0.7	0.505	Tukey-K	0.985
T8	1	Trt*Glucose	Glucose m	G0	G4	-0.98	0.7567	165	-1.3	0.196	Tukey-K	0.785
T8	1	Trt*Glucose	Glucose m	G0	G5	-0.34	0.7567	165	-0.4	0.657	Tukey-K	0.998
T8	1	Trt*Glucose	Glucose m	G1	G2	-0.15	0.7567	165	-0.2	0.848	Tukey-K	1
T8	1	Trt*Glucose	Glucose m	G1	G3	-0.39	0.7567	165	-0.5	0.61	Tukey-K	0.996
T8	1	Trt*Glucose	Glucose m	G1	G4	-0.86	0.7567	165	-1.1	0.256	Tukey-K	0.864

gene	t No	Effect	Slice	Trt	Trt	Estima	Std Err	DF	t val	Raw p	Adju m	Adj p
T8	1	Trt*Glucose	Glucose m	G1	G5	-0.22	0.7567	165	-0.3	0.774	Tukey-K	1
T8	1	Trt*Glucose	Glucose m	G2	G3	-0.24	0.7567	165	-0.3	0.75	Tukey-K	1
T8	1	Trt*Glucose	Glucose m	G2	G4	-0.72	0.7567	165	-0.9	0.344	Tukey-K	0.933
T8	1	Trt*Glucose	Glucose m	G2	G5	-0.07	0.7567	165	-0.1	0.924	Tukey-K	1
T8	1	Trt*Glucose	Glucose m	G3	G4	-0.48	0.7567	165	-0.6	0.529	Tukey-K	0.989
T8	1	Trt*Glucose	Glucose m	G3	G5	0.169	0.7567	165	0.22	0.824	Tukey-K	1
T8	1	Trt*Glucose	Glucose m	G4	G5	0.646	0.7567	165	0.85	0.395	Tukey-K	0.957
T8	1	Trt*Glucose	Glucose p	G0	G1	0.391	0.7567	165	0.52	0.606	Tukey-K	0.995
T8	1	Trt*Glucose	Glucose p	G0	G2	0.444	0.7567	165	0.59	0.559	Tukey-K	0.992
T8	1	Trt*Glucose	Glucose p	G0	G3	1.243	0.7567	165	1.64	0.102	Tukey-K	0.571
T8	1	Trt*Glucose	Glucose p	G0	G4	0.102	0.7567	165	0.13	0.893	Tukey-K	1
T8	1	Trt*Glucose	Glucose p	G0	G5	-0.08	0.7567	165	-0.1	0.911	Tukey-K	1
T8	1	Trt*Glucose	Glucose p	G1	G2	0.053	0.7567	165	0.07	0.944	Tukey-K	1
T8	1	Trt*Glucose	Glucose p	G1	G3	0.853	0.7567	165	1.13	0.261	Tukey-K	0.87
T8	1	Trt*Glucose	Glucose p	G1	G4	-0.29	0.7567	165	-0.4	0.703	Tukey-K	0.999
T8	1	Trt*Glucose	Glucose p	G1	G5	-0.48	0.7567	165	-0.6	0.531	Tukey-K	0.989
T8	1	Trt*Glucose	Glucose p	G2	G3	0.8	0.7567	165	1.06	0.292	Tukey-K	0.897
T8	1	Trt*Glucose	Glucose p	G2	G4	-0.34	0.7567	165	-0.5	0.652	Tukey-K	0.998
T8	1	Trt*Glucose	Glucose p	G2	G5	-0.53	0.7567	165	-0.7	0.486	Tukey-K	0.982
T8	1	Trt*Glucose	Glucose p	G3	G4	-1.14	0.7567	165	-1.5	0.133	Tukey-K	0.659
T8	1	Trt*Glucose	Glucose p	G3	G5	-1.33	0.7567	165	-1.8	0.081	Tukey-K	0.498
T8	1	Trt*Glucose	Glucose p	G4	G5	-0.19	0.7567	165	-0.2	0.806	Tukey-K	1
T9	1	Trt*Glucose	Glucose m	G0	G1	0.101	0.6863	165	0.15	0.883	Tukey-K	1
T9	1	Trt*Glucose	Glucose m	G0	G2	0.029	0.6863	165	0.04	0.966	Tukey-K	1
T9	1	Trt*Glucose	Glucose m	G0	G3	-0.12	0.6863	165	-0.2	0.861	Tukey-K	1
T9	1	Trt*Glucose	Glucose m	G0	G4	-0.6	0.6863	165	-0.9	0.38	Tukey-K	0.951
T9	1	Trt*Glucose	Glucose m	G0	G5	0.475	0.6863	165	0.69	0.49	Tukey-K	0.983
T9	1	Trt*Glucose	Glucose m	G1	G2	-0.07	0.6863	165	-0.1	0.917	Tukey-K	1
T9	1	Trt*Glucose	Glucose m	G1	G3	-0.22	0.6863	165	-0.3	0.747	Tukey-K	1
T9	1	Trt*Glucose	Glucose m	G1	G4	-0.71	0.6863	165	-1	0.306	Tukey-K	0.908
T9	1	Trt*Glucose	Glucose m	G1	G5	0.374	0.6863	165	0.55	0.586	Tukey-K	0.994
T9	1	Trt*Glucose	Glucose m	G2	G3	-0.15	0.6863	165	-0.2	0.827	Tukey-K	1
T9	1	Trt*Glucose	Glucose m	G2	G4	-0.63	0.6863	165	-0.9	0.357	Tukey-K	0.94
T9	1	Trt*Glucose	Glucose m	G2	G5	0.446	0.6863	165	0.65	0.516	Tukey-K	0.987
T9	1	Trt*Glucose	Glucose m	G3	G4	-0.48	0.6863	165	-0.7	0.482	Tukey-K	0.981
T9	1	Trt*Glucose	Glucose m	G3	G5	0.596	0.6863	165	0.87	0.386	Tukey-K	0.953
T9	1	Trt*Glucose	Glucose m	G4	G5	1.08	0.6863	165	1.57	0.118	Tukey-K	0.617
T9	1	Trt*Glucose	Glucose p	G0	G1	-0.08	0.6863	165	-0.1	0.909	Tukey-K	1
T9	1	Trt*Glucose	Glucose p	G0	G2	0.506	0.6863	165	0.74	0.462	Tukey-K	0.977
T9	1	Trt*Glucose	Glucose p	G0	G3	0.294	0.6863	165	0.43	0.669	Tukey-K	0.998
T9	1	Trt*Glucose	Glucose p	G0	G4	-0.34	0.6863	165	-0.5	0.618	Tukey-K	0.996
T9	1	Trt*Glucose	Glucose p	G0	G5	-0.31	0.6863	165	-0.4	0.657	Tukey-K	0.998

gene	No	Effect	Slice	Trt	Trt	Estima	Std Err	DF	t val	Raw p	Adju m	Adj p
T9	1	Trt*Glucose	Glucose p	G1	G2	0.584	0.6863	165	0.85	0.396	Tukey-K	0.957
T9	1	Trt*Glucose	Glucose p	G1	G3	0.372	0.6863	165	0.54	0.589	Tukey-K	0.994
T9	1	Trt*Glucose	Glucose p	G1	G4	-0.26	0.6863	165	-0.4	0.701	Tukey-K	0.999
T9	1	Trt*Glucose	Glucose p	G1	G5	-0.23	0.6863	165	-0.3	0.741	Tukey-K	0.999
T9	1	Trt*Glucose	Glucose p	G2	G3	-0.21	0.6863	165	-0.3	0.757	Tukey-K	1
T9	1	Trt*Glucose	Glucose p	G2	G4	-0.85	0.6863	165	-1.2	0.218	Tukey-K	0.818
T9	1	Trt*Glucose	Glucose p	G2	G5	-0.81	0.6863	165	-1.2	0.239	Tukey-K	0.844
T9	1	Trt*Glucose	Glucose p	G3	G4	-0.64	0.6863	165	-0.9	0.355	Tukey-K	0.939
T9	1	Trt*Glucose	Glucose p	G3	G5	-0.6	0.6863	165	-0.9	0.384	Tukey-K	0.952
T9	1	Trt*Glucose	Glucose p	G4	G5	0.037	0.6863	165	0.05	0.957	Tukey-K	1

TEST 3: Slice comparisons or interaction comparisons between the ages:

Gene	StmtNo	Effect	Slice	AgeCat	_AgeCat	Estimate	StdErr	DF	tValue	Rawp	Adjustment	Adjp
T1	2	AgeCat*Gluc	Glu m	Middle	Old	1.3	1.01	16.6	1.285	0.217	Tukey-K	0.41
T1	2	AgeCat*Gluc	Glu m	Middle	Young	-0.1	1.07	16.6	-0.1	0.923	Tukey-K	0.99
T1	2	AgeCat*Gluc	Glu m	Old	Young	-1.4	0.89	16.6	-1.57	0.134	Tukey-K	0.26
T1	2	AgeCat*Glucose	Glu p	Middle	Old	0.81	1.01	16.6	0.805	0.432	Tukey-K	0.7
T1	2	AgeCat*Glucose	Glu p	Middle	Young	-0.17	1.07	16.6	-0.16	0.872	Tukey-K	0.99
T1	2	AgeCat*Glucose	Glu p	Old	Young	-0.99	0.89	16.6	-1.11	0.283	Tukey-K	0.51
T10	2	AgeCat*Glucose	Glu m	Middle	Old	-0.54	1.36	16.6	-0.4	0.696	Tukey-K	0.92
T10	2	AgeCat*Glucose	Glu m	Middle	Young	-1.66	1.43	16.6	-1.16	0.263	Tukey-K	0.48
T10	2	AgeCat*Glucose	Glu m	Old	Young	-1.12	1.2	16.6	-0.93	0.363	Tukey-K	0.62
T10	2	AgeCat*Glucose	Glu p	Middle	Old	-0.53	1.36	16.6	-0.39	0.704	Tukey-K	0.92
T10	2	AgeCat*Glucose	Glu p	Middle	Young	-1.99	1.43	16.6	-1.39	0.184	Tukey-K	0.35
T10	2	AgeCat*Glucose	Glu p	Old	Young	-1.46	1.2	16.6	-1.22	0.24	Tukey-K	0.44
T2	2	AgeCat*Glucose	Glu m	Middle	Old	1.46	0.94	17.1	1.554	0.139	Tukey-K	0.27
T2	2	AgeCat*Glucose	Glu m	Middle	Young	0.04	0.99	17.1	0.041	0.968	Tukey-K	1
T2	2	AgeCat*Glucose	Glu m	Old	Young	-1.42	0.83	17.1	-1.71	0.105	Tukey-K	0.2
T2	2	AgeCat*Glucose	Glu p	Middle	Old	0.72	0.94	17.1	0.77	0.452	Tukey-K	0.72
T2	2	AgeCat*Glucose	Glu p	Middle	Young	-0.18	0.99	17.1	-0.18	0.858	Tukey-K	0.98
T2	2	AgeCat*Glucose	Glu p	Old	Young	-0.91	0.83	17.1	-1.09	0.291	Tukey-K	0.52
T3	2	AgeCat*Glucose	Glu m	Middle	Old	1.78	0.89	17.2	1.997	0.062	Tukey-K	0.12
T3	2	AgeCat*Glucose	Glu m	Middle	Young	-0.67	0.94	17.2	-0.71	0.488	Tukey-K	0.76
T3	2	AgeCat*Glucose	Glu m	Old	Young	-2.44	0.79	17.2	-3.11	0.006	Tukey-K	0.01
T3	2	AgeCat*Glucose	Glu p	Middle	Old	0.84	0.89	17.2	0.944	0.358	Tukey-K	0.61
T3	2	AgeCat*Glucose	Glu p	Middle	Young	-0.69	0.94	17.2	-0.74	0.471	Tukey-K	0.74
T3	2	AgeCat*Glucose	Glu p	Old	Young	-1.53	0.79	17.2	-1.95	0.067	Tukey-K	0.13
T4	2	AgeCat*Glucose	Glu m	Middle	Old	0.43	0.67	19.8	0.643	0.527	Tukey-K	0.8
T4	2	AgeCat*Glucose	Glu m	Middle	Young	-0.08	0.71	19.8	-0.11	0.917	Tukey-K	0.99
T4	2	AgeCat*Glucose	Glu m	Old	Young	-0.51	0.59	19.8	-0.86	0.402	Tukey-K	0.67
T4	2	AgeCat*Glucose	Glu p	Middle	Old	0.2	0.67	19.8	0.291	0.774	Tukey-K	0.95
T4	2	AgeCat*Glucose	Glu p	Middle	Young	-0.12	0.71	19.8	-0.17	0.865	Tukey-K	0.98
T4	2	AgeCat*Glucose	Glu p	Old	Young	-0.32	0.59	19.8	-0.53	0.599	Tukey-K	0.85
T5	2	AgeCat*Glucose	Glu m	Middle	Old	-1.14	1.99	15.5	-0.57	0.574	Tukey-K	0.83
T5	2	AgeCat*Glucose	Glu m	Middle	Young	0.28	2.09	15.5	0.135	0.895	Tukey-K	0.99
T5	2	AgeCat*Glucose	Glu m	Old	Young	1.42	1.75	15.5	0.811	0.429	Tukey-K	0.7
T5	2	AgeCat*Glucose	Glu p	Middle	Old	-1.77	1.99	15.5	-0.89	0.388	Tukey-K	0.65
T5	2	AgeCat*Glucose	Glu p	Middle	Young	-0.38	2.09	15.5	-0.18	0.857	Tukey-K	0.98
T5	2	AgeCat*Glucose	Glu p	Old	Young	1.38	1.75	15.5	0.789	0.442	Tukey-K	0.71
T6	2	AgeCat*Glucose	Glu m	Middle	Old	0.94	1.97	16.3	0.477	0.639	Tukey-K	0.88
T6	2	AgeCat*Glucose	Glu m	Middle	Young	1	2.08	16.3	0.484	0.635	Tukey-K	0.88
T6	2	AgeCat*Glucose	Glu m	Old	Young	0.06	1.74	16.3	0.037	0.971	Tukey-K	1

Gene	StmtNo	Effect	Slice	AgeCat	_AgeCat	Estimate	StdErr	DF	tValue	Rawp	Adjustment	Adjp
T6	2	AgeCat*Glucose	Glu p	Middle	Old	1.17	1.97	16.3	0.597	0.559	Tukey-K	0.82
T6	2	AgeCat*Glucose	Glu p	Middle	Young	1.92	2.08	16.3	0.923	0.369	Tukey-K	0.63
T6	2	AgeCat*Glucose	Glu p	Old	Young	0.74	1.74	16.3	0.427	0.675	Tukey-K	0.9
T7	2	AgeCat*Glucose	Glu m	Middle	Old	1.95	1.2	17.8	1.629	0.121	Tukey-K	0.24
T7	2	AgeCat*Glucose	Glu m	Middle	Young	0.12	1.26	17.8	0.095	0.925	Tukey-K	1
T7	2	AgeCat*Glucose	Glu m	Old	Young	-1.83	1.06	17.8	-1.73	0.1	Tukey-K	0.2
T7	2	AgeCat*Glucose	Glu p	Middle	Old	1.56	1.2	17.8	1.301	0.21	Tukey-K	0.4
T7	2	AgeCat*Glucose	Glu p	Middle	Young	-0.32	1.26	17.8	-0.25	0.803	Tukey-K	0.97
T7	2	AgeCat*Glucose	Glu p	Old	Young	-1.88	1.06	17.8	-1.78	0.093	Tukey-K	0.18
T8	2	AgeCat*Glucose	Glu m	Middle	Old	-0.19	2	16.2	-0.1	0.925	Tukey-K	0.99
T8	2	AgeCat*Glucose	Glu m	Middle	Young	1.3	2.1	16.2	0.619	0.545	Tukey-K	0.81
T8	2	AgeCat*Glucose	Glu m	Old	Young	1.49	1.76	16.2	0.848	0.409	Tukey-K	0.67
T8	2	AgeCat*Glucose	Glu p	Middle	Old	-0.7	2	16.2	-0.35	0.731	Tukey-K	0.93
T8	2	AgeCat*Glucose	Glu p	Middle	Young	0.23	2.1	16.2	0.112	0.912	Tukey-K	0.99
T8	2	AgeCat*Glucose	Glu p	Old	Young	0.93	1.76	16.2	0.53	0.603	Tukey-K	0.86
T9	2	AgeCat*Glucose	Glu m	Middle	Old	1.78	2.35	15.7	0.755	0.461	Tukey-K	0.73
T9	2	AgeCat*Glucose	Glu m	Middle	Young	1.91	2.48	15.7	0.769	0.453	Tukey-K	0.72
T9	2	AgeCat*Glucose	Glu m	Old	Young	0.13	2.07	15.7	0.063	0.951	Tukey-K	1
T9	2	AgeCat*Glucose	Glu p	Middle	Old	2.87	2.35	15.7	1.221	0.24	Tukey-K	0.44
T9	2	AgeCat*Glucose	Glu p	Middle	Young	1.43	2.48	15.7	0.575	0.573	Tukey-K	0.83
T9	2	AgeCat*Glucose	Glu p	Old	Young	-1.45	2.07	15.7	-0.7	0.496	Tukey-K	0.77

**TEST 4:** Gave the differences between Age, Treatment, and +/- FBS, 30 nM glucose in specific age and treatment:

	No	Effect	AgeCat	Trt	Trt	Estimate	Std Er	DF	tValue	Rawp	Adjustment	Adjp	
T1	3	AgeCat*Trt*Gluco	Middle Gluco m	G0	G1	0.004	0.9	165	0	0.997	Tukey-K	1	
T1	3	AgeCat*Trt*Gluco	Middle Gluco m	G0	G2	0.196	0.9	165	0.22	0.828	Tukey-K	1	
T1	3	AgeCat*Trt*Gluco	Middle Gluco m	G0	G3	-0.06	0.9	165	-0.1	0.945	Tukey-K	1	
T1	3	AgeCat*Trt*Gluco	Middle Gluco m	G0	G4	-0.71	0.9	165	-0.8	0.431	Tukey-K	0.969	
T1	3	AgeCat*Trt*Gluco	Middle Gluco m	G0	G5	0.499	0.9	165	0.55	0.58	Tukey-K	0.994	
T1	3	AgeCat*Trt*Gluco	Middle Gluco m	G1	G2	0.193	0.9	165	0.21	0.831	Tukey-K	1	
T1	3	AgeCat*Trt*Gluco	Middle Gluco m	G1	G3	-0.07	0.9	165	-0.1	0.942	Tukey-K	1	
T1	3	AgeCat*Trt*Gluco	Middle Gluco m	G1	G4	-0.71	0.9	165	-0.8	0.428	Tukey-K	0.968	
T1	3	AgeCat*Trt*Gluco	Middle Gluco m	G1	G5	0.495	0.9	165	0.55	0.583	Tukey-K	0.994	
T1	3	AgeCat*Trt*Gluco	Middle Gluco m	G2	G3	-0.26	0.9	165	-0.3	0.774	Tukey-K	1	
T1	3	AgeCat*Trt*Gluco	Middle Gluco m	G2	G4	-0.91	0.9	165	-1	0.315	Tukey-K	0.915	
T1	3	AgeCat*Trt*Gluco	Middle Gluco m	G2	G5	0.303	0.9	165	0.34	0.737	Tukey-K	0.999	
T1	3	AgeCat*Trt*Gluco	Middle Gluco m	G3	G4	-0.65	0.9	165	-0.7	0.472	Tukey-K	0.979	
T1	3	AgeCat*Trt*Gluco	Middle Gluco m	G3	G5	0.561	0.9	165	0.62	0.534	Tukey-K	0.989	
T1	3	AgeCat*Trt*Glucose	Middle Gluco m	G4	G5	1.209	0.9	165	1.34	0.181	Tukey-K	0.759	
T1	3	AgeCat*Trt*Glucose	Middle Gluco p	G0	G1	1.108	0.9	165	1.23	0.22	Tukey-K	0.821	
T1	3	AgeCat*Trt*Glucose	Middle Gluco p	G0	G2	-0.48	0.9	165	-0.5	0.591	Tukey-K	0.994	
T1	3	AgeCat*Trt*Glucose	Middle Gluco p	G0	G3	-1.34	0.9	165	-1.5	0.138	Tukey-K	0.672	
T1	3	AgeCat*Trt*Glucose	Middle Gluco p	G0	G4	0.191	0.9	165	0.21	0.832	Tukey-K	1	
T1	3	AgeCat*Trt*Glucose	Middle Gluco p	G0	G5	-0.85	0.9	165	-1	0.343	Tukey-K	0.933	
T1	3	AgeCat*Trt*Glucose	Middle Gluco p	G1	G2	-1.59	0.9	165	-1.8	0.078	Tukey-K	0.488	
T1	3	AgeCat*Trt*Glucose	Middle Gluco p	G1	G3	-2.45	0.9	165	-2.7	0.007	Tukey-K	0.076	
T1	3	AgeCat*Trt*Glucose	Middle Gluco p	G1	G4	-0.92	0.9	165	-1	0.31	Tukey-K	0.911	
T1	3	AgeCat*Trt*Glucose	Middle Gluco p	G1	G5	-1.96	0.9	165	-2.2	0.031	Tukey-K	0.252	
T1	3	AgeCat*Trt*Glucose	Middle Gluco p	G2	G3	-0.85	0.9	165	-1	0.343	Tukey-K	0.932	
T1	3	AgeCat*Trt*Glucose	Middle Gluco p	G2	G4	0.676	0.9	165	0.75	0.453	Tukey-K	0.975	
T1	3	AgeCat*Trt*Glucose	Middle Glu p	G2	G5	-0.37	0.9	165	-0.4	0.681	Tukey-K	0.998	
T1	3	AgeCat*Trt*Glucose	Middle Gluco p	G3	G4	1.531	0.9	165	1.7	0.091	Tukey-K	0.532	
T1	3	AgeCat*Trt*Glucose	Middle Gluco p	G3	G5	0.485	0.9	165	0.54	0.59	Tukey-K	0.994	
T1	3	AgeCat*Trt*Glucose	Middle Gluco p	G4	G5	-1.05	0.9	165	-1.2	0.247	Tukey-K	0.854	
T1	3	AgeCat*Trt*Glucose	Old	Gluc m	G0	G1	-0.29	0.6	165	-0.5	0.653	Tukey-K	0.998
T1	3	AgeCat*Trt*Glucose	Old	Gluc m	G0	G2	-0.5	0.6	165	-0.8	0.429	Tukey-K	0.968
T1	3	AgeCat*Trt*Glucose	Old	Gluc m	G0	G3	-0.72	0.6	165	-1.1	0.259	Tukey-K	0.867
T1	3	AgeCat*Trt*Glucose	Old	Gluc m	G0	G4	-1.32	0.6	165	-2.1	0.04	Tukey-K	0.306
T1	3	AgeCat*Trt*Glucose	Old	Gluc m	G0	G5	-0.18	0.6	165	-0.3	0.774	Tukey-K	1
T1	3	AgeCat*Trt*Glucose	Old	Gluc m	G1	G2	-0.22	0.6	165	-0.3	0.732	Tukey-K	0.999
T1	3	AgeCat*Trt*Glucose	Old	Gluc m	G1	G3	-0.43	0.6	165	-0.7	0.497	Tukey-K	0.984

T1	3	AgeCat*Trt*Glucose	Old	Gluc m	G1 G4	-1.03	0.6	165	-1.6	0.106	Tukey-K	0.584
T1	3	AgeCat*Trt*Glucose	Old	Gluc m	G1 G5	0.104	0.6	165	0.16	0.871	Tukey-K	1
T1	3	AgeCat*Trt*Glucose	Old	Gluc m	G2 G3	-0.22	0.6	165	-0.3	0.736	Tukey-K	0.999
T1	3	AgeCat*Trt*Glucose	Old	Gluc m	G2 G4	-0.81	0.6	165	-1.3	0.202	Tukey-K	0.795
T1	3	AgeCat*Trt*Glucose	Old	Gluc m	G2 G5	0.322	0.6	165	0.51	0.613	Tukey-K	0.996
T1	3	AgeCat*Trt*Glucose	Old	Gluc m	G3 G4	-0.6	0.6	165	-0.9	0.347	Tukey-K	0.935
T1	3	AgeCat*Trt*Glucose	Old	Gluc m	G3 G5	0.537	0.6	165	0.84	0.4	Tukey-K	0.959
T1	3	AgeCat*Trt*Glucose	Old	Glu m	G4 G5	1.137	0.6	165	1.79	0.076	Tukey-K	0.477
T1	3	AgeCat*Trt*Glucose	Old	Glu m	G0 G1	-0.07	0.6	165	-0.1	0.918	Tukey-K	1
T1	3	AgeCat*Trt*Glucose	Old	Glu p	G0 G2	-0.29	0.6	165	-0.5	0.646	Tukey-K	0.997
T1	3	AgeCat*Trt*Glucose	Old	Glu p	G0 G3	-0.07	0.6	165	-0.1	0.915	Tukey-K	1
T1	3	AgeCat*Trt*Glucose	Old	Glu p	G0 G4	-1.01	0.6	165	-1.6	0.116	Tukey-K	0.613
T1	3	AgeCat*Trt*Glucose	Old	Glu p	G0 G5	-0.33	0.6	165	-0.5	0.604	Tukey-K	0.995
T1	3	AgeCat*Trt*Glucose	Old	Glu p	G1 G2	-0.23	0.6	165	-0.4	0.722	Tukey-K	0.999
T1	3	AgeCat*Trt*Glucose	Old	Glu p	G1 G3	-0	0.6	165	-0	0.998	Tukey-K	1
T1	3	AgeCat*Trt*Glucose	Old	Glu p	G1 G4	-0.94	0.6	165	-1.5	0.142	Tukey-K	0.68
T1	3	AgeCat*Trt*Glucose	Old	Glu p	G1 G5	-0.26	0.6	165	-0.4	0.678	Tukey-K	0.998
T1	3	AgeCat*Trt*Glucose	Old	Glu p	G2 G3	0.225	0.6	165	0.35	0.724	Tukey-K	0.999
T1	3	AgeCat*Trt*Glucose	Old	Glu p	G2 G4	-0.71	0.6	165	-1.1	0.264	Tukey-K	0.873
T1	3	AgeCat*Trt*Glucose	Old	Glu p	G2 G5	-0.04	0.6	165	-0.1	0.953	Tukey-K	1
T1	3	AgeCat*Trt*Glucose	Old	Glu p	G3 G4	-0.94	0.6	165	-1.5	0.142	Tukey-K	0.681
T1	3	AgeCat*Trt*Glucose	Old	Glu p	G3 G5	-0.26	0.6	165	-0.4	0.68	Tukey-K	0.998
T1	3	AgeCat*Trt*Glucose	Old	Glu p	G4 G5	0.675	0.6	165	1.06	0.29	Tukey-K	0.896
T1	3	AgeCat*Trt*Glucose	Young	Glu m	G0 G1	0.088	0.7	165	0.12	0.905	Tukey-K	1
T1	3	AgeCat*Trt*Glucose	Young	Glu m	G0 G2	0.403	0.7	165	0.55	0.584	Tukey-K	0.994
T1	3	AgeCat*Trt*Glucose	Young	Glu m	G0 G3	-0.3	0.7	165	-0.4	0.685	Tukey-K	0.999
T1	3	AgeCat*Trt*Glucose	Young	Glu m	G0 G4	0.838	0.7	165	1.14	0.256	Tukey-K	0.864
T1	3	AgeCat*Trt*Glucose	Young	Glu m	G0 G5	0.22	0.7	165	0.3	0.765	Tukey-K	1
T1	3	AgeCat*Trt*Glucose	Young	Glu m	G1 G2	0.315	0.7	165	0.43	0.668	Tukey-K	0.998
T1	3	AgeCat*Trt*Glucose	Young	Glu m	G1 G3	-0.39	0.7	165	-0.5	0.599	Tukey-K	0.995
T1	3	AgeCat*Trt*Glucose	Young	Glu m	G1 G4	0.75	0.7	165	1.02	0.309	Tukey-K	0.91
T1	3	AgeCat*Trt*Glucose	Young	Glu m	G1 G5	0.132	0.7	165	0.18	0.857	Tukey-K	1
T1	3	AgeCat*Trt*Glucose	Young	Glu m	G2 G3	-0.7	0.7	165	-1	0.34	Tukey-K	0.931
T1	3	AgeCat*Trt*Glucose	Young	Glu m	G2 G4	0.434	0.7	165	0.59	0.555	Tukey-K	0.992
T1	3	AgeCat*Trt*Glucose	Young	Glu m	G2 G5	-0.18	0.7	165	-0.2	0.803	Tukey-K	1
T1	3	AgeCat*Trt*Glucose	Young	Glu m	G3 G4	1.136	0.7	165	1.55	0.124	Tukey-K	0.634
T1	3	AgeCat*Trt*Glucose	Young	Glu m	G3 G5	0.519	0.7	165	0.71	0.481	Tukey-K	0.981
T1	3	AgeCat*Trt*Glucose	Young	Glu m	G4 G5	-0.62	0.7	165	-0.8	0.402	Tukey-K	0.959
T1	3	AgeCat*Trt*Glucose	Young	Glu p	G0 G1	1.236	0.7	165	1.68	0.094	Tukey-K	0.545
T1	3	AgeCat*Trt*Glucose	Young	Glu p	G0 G2	0.545	0.7	165	0.74	0.459	Tukey-K	0.976
T1	3	AgeCat*Trt*Glucose	Young	Glu p	G0 G3	0.877	0.7	165	1.19	0.234	Tukey-K	0.839

T1	3	AgeCat*Trt*Glucose	Young Glu p	G0 G4	1.006	0.7	165	1.37	0.173	Tukey-K	0.745
T1	3	AgeCat*Trt*Glucose	Young Glu p	G0 G5	0.598	0.7	165	0.81	0.416	Tukey-K	0.964
T1	3	AgeCat*Trt*Glucose	Young Glu p	G1 G2	-0.69	0.7	165	-0.9	0.348	Tukey-K	0.935
T1	3	AgeCat*Trt*Glucose	Young Glu p	G1 G3	-0.36	0.7	165	-0.5	0.625	Tukey-K	0.996
T1	3	AgeCat*Trt*Glucose	Young Glu p	G1 G4	-0.23	0.7	165	-0.3	0.754	Tukey-K	1
T1	3	AgeCat*Trt*Glucose	Young Glu p	G1 G5	-0.64	0.7	165	-0.9	0.386	Tukey-K	0.953
T1	3	AgeCat*Trt*Glucose	Young Glu p	G2 G3	0.331	0.7	165	0.45	0.652	Tukey-K	0.998
T1	3	AgeCat*Trt*Glucose	Young Glu p	G2 G4	0.46	0.7	165	0.63	0.531	Tukey-K	0.989
T1	3	AgeCat*Trt*Glucose	Young Glu p	G2 G5	0.053	0.7	165	0.07	0.943	Tukey-K	1
T1	3	AgeCat*Trt*Glucose	Young Glu p	G3 G4	0.129	0.7	165	0.18	0.861	Tukey-K	1
T1	3	AgeCat*Trt*Glucose	Young Glu p	G3 G5	-0.28	0.7	165	-0.4	0.705	Tukey-K	0.999
T1	3	AgeCat*Trt*Glucose	Young Glu p	G4 G5	-0.41	0.7	165	-0.6	0.58	Tukey-K	0.994
T10	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G1	-0.86	1.2	165	-0.7	0.481	Tukey-K	0.981
T10	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G2	-0.82	1.2	165	-0.7	0.5	Tukey-K	0.984
T10	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G3	-0.86	1.2	165	-0.7	0.48	Tukey-K	0.981
T10	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G4	-1.87	1.2	165	-1.5	0.123	Tukey-K	0.633
T10	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G5	-0.5	1.2	165	-0.4	0.681	Tukey-K	0.998
T10	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G2	0.037	1.2	165	0.03	0.975	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G3	-0	1.2	165	-0	1	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G4	-1.02	1.2	165	-0.8	0.401	Tukey-K	0.959
T10	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G5	0.357	1.2	165	0.3	0.768	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G3	-0.04	1.2	165	-0	0.975	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G4	-1.06	1.2	165	-0.9	0.384	Tukey-K	0.952
T10	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G5	0.32	1.2	165	0.26	0.792	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Middle Glu m	G3 G4	-1.02	1.2	165	-0.8	0.401	Tukey-K	0.959
T10	3	AgeCat*Trt*Glucose	Middle Glu m	G3 G5	0.358	1.2	165	0.3	0.768	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Middle Glu m	G4 G5	1.377	1.2	165	1.14	0.257	Tukey-K	0.865
T10	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G1	0.882	1.2	165	0.73	0.467	Tukey-K	0.978
T10	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G2	0.776	1.2	165	0.64	0.522	Tukey-K	0.988
T10	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G3	-0.47	1.2	165	-0.4	0.7	Tukey-K	0.999
T10	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G4	1.331	1.2	165	1.1	0.273	Tukey-K	0.881
T10	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G5	-0.17	1.2	165	-0.1	0.89	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G2	-0.11	1.2	165	-0.1	0.93	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G3	-1.35	1.2	165	-1.1	0.267	Tukey-K	0.875
T10	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G4	0.449	1.2	165	0.37	0.711	Tukey-K	0.999
T10	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G5	-1.05	1.2	165	-0.9	0.387	Tukey-K	0.954
T10	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G3	-1.24	1.2	165	-1	0.306	Tukey-K	0.908
T10	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G4	0.555	1.2	165	0.46	0.647	Tukey-K	0.997
T10	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G5	-0.94	1.2	165	-0.8	0.437	Tukey-K	0.971
T10	3	AgeCat*Trt*Glucose	Middle Glu p	G3 G4	1.798	1.2	165	1.49	0.139	Tukey-K	0.674

T10	3	AgeCat*Trt*Glucose	Middle Glu p	G3 G5	0.3	1.2	165	0.25	0.804	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Middle Glu p	G4 G5	-1.5	1.2	165	-1.2	0.218	Tukey-K	0.818
T10	3	AgeCat*Trt*Glucose	Old Glu m	G0 G1	0.91	0.9	165	1.06	0.289	Tukey-K	0.895
T10	3	AgeCat*Trt*Glucose	Old Glu m	G0 G2	-1.01	0.9	165	-1.2	0.239	Tukey-K	0.845
T10	3	AgeCat*Trt*Glucose	Old Glu m	G0 G3	-1.4	0.9	165	-1.6	0.103	Tukey-K	0.574
T10	3	AgeCat*Trt*Glucose	Old Glu m	G0 G4	-1.12	0.9	165	-1.3	0.193	Tukey-K	0.78
T10	3	AgeCat*Trt*Glucose	Old Glu m	G0 G5	-0.98	0.9	165	-1.1	0.252	Tukey-K	0.86
T10	3	AgeCat*Trt*Glucose	Old Glu m	G1 G2	-1.92	0.9	165	-2.2	0.026	Tukey-K	0.223
T10	3	AgeCat*Trt*Glucose	Old Glu m	G1 G3	-2.31	0.9	165	-2.7	0.008	Tukey-K	0.08
T10	3	AgeCat*Trt*Glucose	Old Glu m	G1 G4	-2.03	0.9	165	-2.4	0.019	Tukey-K	0.173
T10	3	AgeCat*Trt*Glucose	Old Glu m	G1 G5	-1.89	0.9	165	-2.2	0.028	Tukey-K	0.238
T10	3	AgeCat*Trt*Glucose	Old Glu m	G2 G3	-0.39	0.9	165	-0.5	0.649	Tukey-K	0.997
T10	3	AgeCat*Trt*Glucose	Old Glu m	G2 G4	-0.11	0.9	165	-0.1	0.9	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Old Glu m	G2 G5	0.029	0.9	165	0.03	0.973	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Old Glu m	G3 G4	0.283	0.9	165	0.33	0.741	Tukey-K	0.999
T10	3	AgeCat*Trt*Glucose	Old Glu m	G3 G5	0.419	0.9	165	0.49	0.625	Tukey-K	0.996
T10	3	AgeCat*Trt*Glucose	Old Glu m	G4 G5	0.136	0.9	165	0.16	0.874	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Old Glu p	G0 G1	1.379	0.9	165	1.61	0.109	Tukey-K	0.592
T10	3	AgeCat*Trt*Glucose	Old Glu p	G0 G2	2.042	0.9	165	2.39	0.018	Tukey-K	0.167
T10	3	AgeCat*Trt*Glucose	Old Glu p	G0 G3	0.687	0.9	165	0.8	0.423	Tukey-K	0.967
T10	3	AgeCat*Trt*Glucose	Old Glu p	G0 G4	-0.26	0.9	165	-0.3	0.761	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Old Glu p	G0 G5	0.778	0.9	165	0.91	0.365	Tukey-K	0.944
T10	3	AgeCat*Trt*Glucose	Old Glu p	G1 G2	0.663	0.9	165	0.77	0.44	Tukey-K	0.971
T10	3	AgeCat*Trt*Glucose	Old Glu p	G1 G3	-0.69	0.9	165	-0.8	0.42	Tukey-K	0.966
T10	3	AgeCat*Trt*Glucose	Old Glu p	G1 G4	-1.64	0.9	165	-1.9	0.057	Tukey-K	0.396
T10	3	AgeCat*Trt*Glucose	Old Glu p	G1 G5	-0.6	0.9	165	-0.7	0.483	Tukey-K	0.981
T10	3	AgeCat*Trt*Glucose	Old Glu p	G2 G3	-1.35	0.9	165	-1.6	0.115	Tukey-K	0.611
T10	3	AgeCat*Trt*Glucose	Old Glu p	G2 G4	-2.3	0.9	165	-2.7	0.008	Tukey-K	0.083
T10	3	AgeCat*Trt*Glucose	Old Glu p	G2 G5	-1.26	0.9	165	-1.5	0.142	Tukey-K	0.679
T10	3	AgeCat*Trt*Glucose	Old Glu p	G3 G4	-0.95	0.9	165	-1.1	0.27	Tukey-K	0.878
T10	3	AgeCat*Trt*Glucose	Old Glu p	G3 G5	0.091	0.9	165	0.11	0.916	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Old Glu p	G4 G5	1.038	0.9	165	1.21	0.227	Tukey-K	0.83
T10	3	AgeCat*Trt*Glucose	Young Glu m	G0 G1	0.638	1	165	0.65	0.519	Tukey-K	0.987
T10	3	AgeCat*Trt*Glucose	Young Glu m	G0 G2	0.003	1	165	0	0.997	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Young Glu m	G0 G3	-0.55	1	165	-0.6	0.58	Tukey-K	0.994
T10	3	AgeCat*Trt*Glucose	Young Glu m	G0 G4	-0.1	1	165	-0.1	0.923	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Young Glu m	G0 G5	0.162	1	165	0.16	0.87	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Young Glu m	G1 G2	-0.63	1	165	-0.6	0.522	Tukey-K	0.988
T10	3	AgeCat*Trt*Glucose	Young Glu m	G1 G3	-1.19	1	165	-1.2	0.232	Tukey-K	0.836
T10	3	AgeCat*Trt*Glucose	Young Glu m	G1 G4	-0.73	1	165	-0.7	0.459	Tukey-K	0.976

T10	3	AgeCat*Trt*Glucose	Young Glu m	G1 G5	-0.48	1	165	-0.5	0.63	Tukey-K	0.997
T10	3	AgeCat*Trt*Glucose	Young Glu m	G2 G3	-0.55	1	165	-0.6	0.578	Tukey-K	0.994
T10	3	AgeCat*Trt*Glucose	Young Glu m	G2 G4	-0.1	1	165	-0.1	0.92	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Young Glu m	G2 G5	0.158	1	165	0.16	0.873	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Young Glu m	G3 G4	0.452	1	165	0.46	0.648	Tukey-K	0.997
T10	3	AgeCat*Trt*Glucose	Young Glu m	G3 G5	0.71	1	165	0.72	0.474	Tukey-K	0.979
T10	3	AgeCat*Trt*Glucose	Young Glu m	G4 G5	0.258	1	165	0.26	0.795	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Young Glu p	G0 G1	0.282	1	165	0.29	0.776	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Young Glu p	G0 G2	-0.1	1	165	-0.1	0.915	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Young Glu p	G0 G3	-0.67	1	165	-0.7	0.497	Tukey-K	0.984
T10	3	AgeCat*Trt*Glucose	Young Glu p	G0 G4	-0.66	1	165	-0.7	0.506	Tukey-K	0.985
T10	3	AgeCat*Trt*Glucose	Young Glu p	G0 G5	-0.96	1	165	-1	0.331	Tukey-K	0.925
T10	3	AgeCat*Trt*Glucose	Young Glu p	G1 G2	-0.39	1	165	-0.4	0.696	Tukey-K	0.999
T10	3	AgeCat*Trt*Glucose	Young Glu p	G1 G3	-0.95	1	165	-1	0.335	Tukey-K	0.928
T10	3	AgeCat*Trt*Glucose	Young Glu p	G1 G4	-0.94	1	165	-1	0.343	Tukey-K	0.932
T10	3	AgeCat*Trt*Glucose	Young Glu p	G1 G5	-1.25	1	165	-1.3	0.209	Tukey-K	0.806
T10	3	AgeCat*Trt*Glucose	Young Glu p	G2 G3	-0.57	1	165	-0.6	0.566	Tukey-K	0.993
T10	3	AgeCat*Trt*Glucose	Young Glu p	G2 G4	-0.55	1	165	-0.6	0.576	Tukey-K	0.993
T10	3	AgeCat*Trt*Glucose	Young Glu p	G2 G5	-0.86	1	165	-0.9	0.386	Tukey-K	0.953
T10	3	AgeCat*Trt*Glucose	Young Glu p	G3 G4	0.014	1	165	0.01	0.989	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Young Glu p	G3 G5	-0.29	1	165	-0.3	0.769	Tukey-K	1
T10	3	AgeCat*Trt*Glucose	Young Glu p	G4 G5	-0.3	1	165	-0.3	0.758	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G1	0.339	1	165	0.35	0.724	Tukey-K	0.999
T2	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G2	0.736	1	165	0.77	0.443	Tukey-K	0.972
T2	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G3	-0.62	1	165	-0.7	0.516	Tukey-K	0.987
T2	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G4	-0.65	1	165	-0.7	0.497	Tukey-K	0.984
T2	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G5	0.176	1	165	0.18	0.854	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G2	0.397	1	165	0.41	0.679	Tukey-K	0.998
T2	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G3	-0.96	1	165	-1	0.316	Tukey-K	0.916
T2	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G4	-0.99	1	165	-1	0.302	Tukey-K	0.906
T2	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G5	-0.16	1	165	-0.2	0.866	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G3	-1.36	1	165	-1.4	0.158	Tukey-K	0.715
T2	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G4	-1.39	1	165	-1.4	0.149	Tukey-K	0.697
T2	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G5	-0.56	1	165	-0.6	0.56	Tukey-K	0.992
T2	3	AgeCat*Trt*Glucose	Middle Glu m	G3 G4	-0.03	1	165	-0	0.976	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Middle Glu m	G3 G5	0.801	1	165	0.84	0.405	Tukey-K	0.961
T2	3	AgeCat*Trt*Glucose	Middle Glu m	G4 G5	0.829	1	165	0.87	0.388	Tukey-K	0.954
T2	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G1	0.712	1	165	0.74	0.459	Tukey-K	0.976
T2	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G2	-1.52	1	165	-1.6	0.115	Tukey-K	0.609
T2	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G3	-1.76	1	165	-1.8	0.068	Tukey-K	0.444
T2	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G4	-0.05	1	165	-0.1	0.956	Tukey-K	1

T2	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G5	-1.19	1	165	-1.2	0.216	Tukey-K	0.815
T2	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G2	-2.23	1	165	-2.3	0.021	Tukey-K	0.188
T2	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G3	-2.47	1	165	-2.6	0.011	Tukey-K	0.108
T2	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G4	-0.77	1	165	-0.8	0.426	Tukey-K	0.967
T2	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G5	-1.9	1	165	-2	0.049	Tukey-K	0.356
T2	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G3	-0.24	1	165	-0.3	0.802	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G4	1.467	1	165	1.53	0.128	Tukey-K	0.645
T2	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G5	0.33	1	165	0.34	0.731	Tukey-K	0.999
T2	3	AgeCat*Trt*Glucose	Middle Glu p	G3 G4	1.708	1	165	1.78	0.077	Tukey-K	0.48
T2	3	AgeCat*Trt*Glucose	Middle Glu p	G3 G5	0.571	1	165	0.6	0.552	Tukey-K	0.991
T2	3	AgeCat*Trt*Glucose	Middle Glu p	G4 G5	-1.14	1	165	-1.2	0.237	Tukey-K	0.843
T2	3	AgeCat*Trt*Glucose	Old Glu m	G0 G1	-0.69	0.7	165	-1	0.309	Tukey-K	0.911
T2	3	AgeCat*Trt*Glucose	Old Glu m	G0 G2	-0.47	0.7	165	-0.7	0.49	Tukey-K	0.983
T2	3	AgeCat*Trt*Glucose	Old Glu m	G0 G3	-0.51	0.7	165	-0.8	0.453	Tukey-K	0.975
T2	3	AgeCat*Trt*Glucose	Old Glu m	G0 G4	-1.46	0.7	165	-2.1	0.033	Tukey-K	0.268
T2	3	AgeCat*Trt*Glucose	Old Glu m	G0 G5	-0.14	0.7	165	-0.2	0.836	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Old Glu m	G1 G2	0.223	0.7	165	0.33	0.742	Tukey-K	0.999
T2	3	AgeCat*Trt*Glucose	Old Glu m	G1 G3	0.182	0.7	165	0.27	0.789	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Old Glu m	G1 G4	-0.76	0.7	165	-1.1	0.261	Tukey-K	0.869
T2	3	AgeCat*Trt*Glucose	Old Glu m	G1 G5	0.551	0.7	165	0.81	0.417	Tukey-K	0.965
T2	3	AgeCat*Trt*Glucose	Old Glu m	G2 G3	-0.04	0.7	165	-0.1	0.952	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Old Glu m	G2 G4	-0.99	0.7	165	-1.5	0.147	Tukey-K	0.692
T2	3	AgeCat*Trt*Glucose	Old Glu m	G2 G5	0.328	0.7	165	0.48	0.629	Tukey-K	0.997
T2	3	AgeCat*Trt*Glucose	Old Glu m	G3 G4	-0.95	0.7	165	-1.4	0.164	Tukey-K	0.729
T2	3	AgeCat*Trt*Glucose	Old Glu m	G3 G5	0.369	0.7	165	0.55	0.586	Tukey-K	0.994
T2	3	AgeCat*Trt*Glucose	Old Glu m	G4 G5	1.316	0.7	165	1.94	0.054	Tukey-K	0.381
T2	3	AgeCat*Trt*Glucose	Old Glu p	G0 G1	-0.63	0.7	165	-0.9	0.355	Tukey-K	0.939
T2	3	AgeCat*Trt*Glucose	Old Glu p	G0 G2	0.231	0.7	165	0.34	0.734	Tukey-K	0.999
T2	3	AgeCat*Trt*Glucose	Old Glu p	G0 G3	0.715	0.7	165	1.06	0.293	Tukey-K	0.898
T2	3	AgeCat*Trt*Glucose	Old Glu p	G0 G4	-0.42	0.7	165	-0.6	0.541	Tukey-K	0.99
T2	3	AgeCat*Trt*Glucose	Old Glu p	G0 G5	0.125	0.7	165	0.18	0.854	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Old Glu p	G1 G2	0.86	0.7	165	1.27	0.206	Tukey-K	0.801
T2	3	AgeCat*Trt*Glucose	Old Glu p	G1 G3	1.344	0.7	165	1.98	0.049	Tukey-K	0.356
T2	3	AgeCat*Trt*Glucose	Old Glu p	G1 G4	0.214	0.7	165	0.32	0.753	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Old Glu p	G1 G5	0.754	0.7	165	1.11	0.267	Tukey-K	0.875
T2	3	AgeCat*Trt*Glucose	Old Glu p	G2 G3	0.484	0.7	165	0.71	0.476	Tukey-K	0.98
T2	3	AgeCat*Trt*Glucose	Old Glu p	G2 G4	-0.65	0.7	165	-1	0.342	Tukey-K	0.932
T2	3	AgeCat*Trt*Glucose	Old Glu p	G2 G5	-0.11	0.7	165	-0.2	0.876	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Old Glu p	G3 G4	-1.13	0.7	165	-1.7	0.097	Tukey-K	0.555
T2	3	AgeCat*Trt*Glucose	Old Glu p	G3 G5	-0.59	0.7	165	-0.9	0.385	Tukey-K	0.953
T2	3	AgeCat*Trt*Glucose	Old Glu p	G4 G5	0.54	0.7	165	0.8	0.426	Tukey-K	0.968

T2	3	AgeCat*Trt*Glucose	Old Glu p	G0 G1	-0.03	0.8	165	-0	0.969	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Old Glu p	G0 G2	1.369	0.8	165	1.75	0.082	Tukey-K	0.501
T2	3	AgeCat*Trt*Glucose	Young Glu m	G0 G3	0.437	0.8	165	0.56	0.577	Tukey-K	0.993
T2	3	AgeCat*Trt*Glucose	Young Glu m	G0 G4	0.972	0.8	165	1.24	0.216	Tukey-K	0.816
T2	3	AgeCat*Trt*Glucose	Young Glu m	G0 G5	0.982	0.8	165	1.25	0.211	Tukey-K	0.809
T2	3	AgeCat*Trt*Glucose	Young Glu m	G1 G2	1.4	0.8	165	1.79	0.076	Tukey-K	0.476
T2	3	AgeCat*Trt*Glucose	Young Glu m	G1 G3	0.467	0.8	165	0.6	0.551	Tukey-K	0.991
T2	3	AgeCat*Trt*Glucose	Young Glu m	G1 G4	1.002	0.8	165	1.28	0.202	Tukey-K	0.795
T2	3	AgeCat*Trt*Glucose	Young Glu m	G1 G5	1.012	0.8	165	1.29	0.198	Tukey-K	0.788
T2	3	AgeCat*Trt*Glucose	Young Glu m	G2 G3	-0.93	0.8	165	-1.2	0.235	Tukey-K	0.841
T2	3	AgeCat*Trt*Glucose	Young Glu m	G2 G4	-0.4	0.8	165	-0.5	0.612	Tukey-K	0.996
T2	3	AgeCat*Trt*Glucose	Young Glu m	G2 G5	-0.39	0.8	165	-0.5	0.621	Tukey-K	0.996
T2	3	AgeCat*Trt*Glucose	Young Glu m	G3 G4	0.535	0.8	165	0.68	0.496	Tukey-K	0.984
T2	3	AgeCat*Trt*Glucose	Young Glu m	G3 G5	0.545	0.8	165	0.7	0.487	Tukey-K	0.982
T2	3	AgeCat*Trt*Glucose	Young Glu m	G4 G5	0.01	0.8	165	0.01	0.99	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Young Glu p	G0 G1	1.225	0.8	165	1.56	0.12	Tukey-K	0.623
T2	3	AgeCat*Trt*Glucose	Young Glu p	G0 G2	1.077	0.8	165	1.38	0.171	Tukey-K	0.741
T2	3	AgeCat*Trt*Glucose	Young Glu p	G0 G3	1.065	0.8	165	1.36	0.175	Tukey-K	0.75
T2	3	AgeCat*Trt*Glucose	Young Glu p	G0 G4	1.267	0.8	165	1.62	0.107	Tukey-K	0.587
T2	3	AgeCat*Trt*Glucose	Young Glu p	G0 G5	0.798	0.8	165	1.02	0.309	Tukey-K	0.911
T2	3	AgeCat*Trt*Glucose	Young Glu p	G1 G2	-0.15	0.8	165	-0.2	0.851	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Young Glu p	G1 G3	-0.16	0.8	165	-0.2	0.839	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Young Glu p	G1 G4	0.043	0.8	165	0.05	0.956	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Young Glu p	G1 G5	-0.43	0.8	165	-0.5	0.587	Tukey-K	0.994
T2	3	AgeCat*Trt*Glucose	Young Glu p	G2 G3	-0.01	0.8	165	-0	0.988	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Young Glu p	G2 G4	0.19	0.8	165	0.24	0.808	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Young Glu p	G2 G5	-0.28	0.8	165	-0.4	0.722	Tukey-K	0.999
T2	3	AgeCat*Trt*Glucose	Young Glu p	G3 G4	0.202	0.8	165	0.26	0.796	Tukey-K	1
T2	3	AgeCat*Trt*Glucose	Young Glu p	G3 G5	-0.27	0.8	165	-0.3	0.734	Tukey-K	0.999
T2	3	AgeCat*Trt*Glucose	Young Glu p	G4 G5	-0.47	0.8	165	-0.6	0.55	Tukey-K	0.991
T3	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G1	0.834	0.9	165	0.9	0.368	Tukey-K	0.945
T3	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G2	0.807	0.9	165	0.87	0.383	Tukey-K	0.952
T3	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G3	0.587	0.9	165	0.64	0.526	Tukey-K	0.988
T3	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G4	-0.31	0.9	165	-0.3	0.741	Tukey-K	0.999
T3	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G5	1.043	0.9	165	1.13	0.26	Tukey-K	0.869
T3	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G2	-0.03	0.9	165	-0	0.977	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G3	-0.25	0.9	165	-0.3	0.789	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G4	-1.14	0.9	165	-1.2	0.219	Tukey-K	0.819
T3	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G5	0.209	0.9	165	0.23	0.821	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G3	-0.22	0.9	165	-0.2	0.811	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G4	-1.11	0.9	165	-1.2	0.23	Tukey-K	0.833

T3	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G5	0.235	0.9	165	0.25	0.799	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Middle Glu m	G3 G4	-0.89	0.9	165	-1	0.335	Tukey-K	0.928
T3	3	AgeCat*Trt*Glucose	Middle Glu m	G3 G5	0.456	0.9	165	0.49	0.622	Tukey-K	0.996
T3	3	AgeCat*Trt*Glucose	Middle Glu m	G4 G5	1.348	0.9	165	1.46	0.146	Tukey-K	0.69
T3	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G1	1.488	0.9	165	1.61	0.109	Tukey-K	0.592
T3	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G2	0.274	0.9	165	0.3	0.767	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G3	-1.88	0.9	165	-2	0.043	Tukey-K	0.327
T3	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G4	0.376	0.9	165	0.41	0.684	Tukey-K	0.999
T3	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G5	-0.6	0.9	165	-0.7	0.514	Tukey-K	0.987
T3	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G2	-1.21	0.9	165	-1.3	0.19	Tukey-K	0.777
T3	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G3	-3.37	0.9	165	-3.6	4E-04	Tukey-K	0.005
T3	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G4	-1.11	0.9	165	-1.2	0.23	Tukey-K	0.834
T3	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G5	-2.09	0.9	165	-2.3	0.025	Tukey-K	0.214
T3	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G3	-2.15	0.9	165	-2.3	0.021	Tukey-K	0.187
T3	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G4	0.102	0.9	165	0.11	0.912	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G5	-0.88	0.9	165	-1	0.343	Tukey-K	0.932
T3	3	AgeCat*Trt*Glucose	Middle Glu p	G3 G4	2.255	0.9	165	2.44	0.016	Tukey-K	0.148
T3	3	AgeCat*Trt*Glucose	Middle Glu p	G3 G5	1.275	0.9	165	1.38	0.169	Tukey-K	0.738
T3	3	AgeCat*Trt*Glucose	Middle Glu p	G4 G5	-0.98	0.9	165	-1.1	0.29	Tukey-K	0.896
T3	3	AgeCat*Trt*Glucose	Old Glu m	G0 G1	0.123	0.7	165	0.19	0.85	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Old Glu m	G0 G2	-0.37	0.7	165	-0.6	0.568	Tukey-K	0.993
T3	3	AgeCat*Trt*Glucose	Old Glu m	G0 G3	-0.66	0.7	165	-1	0.315	Tukey-K	0.915
T3	3	AgeCat*Trt*Glucose	Old Glu m	G0 G4	-0.88	0.7	165	-1.3	0.181	Tukey-K	0.76
T3	3	AgeCat*Trt*Glucose	Old Glu m	G0 G5	-0.19	0.7	165	-0.3	0.767	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Old Glu m	G1 G2	-0.5	0.7	165	-0.8	0.448	Tukey-K	0.974
T3	3	AgeCat*Trt*Glucose	Old Glu m	G1 G3	-0.78	0.7	165	-1.2	0.233	Tukey-K	0.838
T3	3	AgeCat*Trt*Glucose	Old Glu m	G1 G4	-1	0.7	165	-1.5	0.127	Tukey-K	0.643
T3	3	AgeCat*Trt*Glucose	Old Glu m	G1 G5	-0.32	0.7	165	-0.5	0.627	Tukey-K	0.997
T3	3	AgeCat*Trt*Glucose	Old Glu m	G2 G3	-0.28	0.7	165	-0.4	0.664	Tukey-K	0.998
T3	3	AgeCat*Trt*Glucose	Old Glu m	G2 G4	-0.5	0.7	165	-0.8	0.441	Tukey-K	0.972
T3	3	AgeCat*Trt*Glucose	Old Glu m	G2 G5	0.179	0.7	165	0.27	0.784	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Old Glu m	G3 G4	-0.22	0.7	165	-0.3	0.737	Tukey-K	0.999
T3	3	AgeCat*Trt*Glucose	Old Glu m	G3 G5	0.464	0.7	165	0.71	0.478	Tukey-K	0.98
T3	3	AgeCat*Trt*Glucose	Old Glu m	G4 G5	0.683	0.7	165	1.05	0.297	Tukey-K	0.901
T3	3	AgeCat*Trt*Glucose	Old Glu p	G0 G1	-0.11	0.7	165	-0.2	0.866	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Old Glu p	G0 G2	0.078	0.7	165	0.12	0.905	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Old Glu p	G0 G3	0.684	0.7	165	1.05	0.296	Tukey-K	0.901
T3	3	AgeCat*Trt*Glucose	Old Glu p	G0 G4	-0.46	0.7	165	-0.7	0.478	Tukey-K	0.98
T3	3	AgeCat*Trt*Glucose	Old Glu p	G0 G5	-0.05	0.7	165	-0.1	0.942	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Old Glu p	G1 G2	0.188	0.7	165	0.29	0.774	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Old Glu p	G1 G3	0.794	0.7	165	1.22	0.225	Tukey-K	0.828

T3	3	AgeCat*Trt*Glucose	Old Glu p	G1 G4	-0.35	0.7	165	-0.5	0.589	Tukey-K	0.994
T3	3	AgeCat*Trt*Glucose	Old Glu p	G1 G5	0.062	0.7	165	0.1	0.924	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Old Glu p	G2 G3	0.606	0.7	165	0.93	0.354	Tukey-K	0.939
T3	3	AgeCat*Trt*Glucose	Old Glu p	G2 G4	-0.54	0.7	165	-0.8	0.408	Tukey-K	0.962
T3	3	AgeCat*Trt*Glucose	Old Glu p	G2 G5	-0.13	0.7	165	-0.2	0.848	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Old Glu p	G3 G4	-1.15	0.7	165	-1.8	0.081	Tukey-K	0.495
T3	3	AgeCat*Trt*Glucose	Old Glu p	G3 G5	-0.73	0.7	165	-1.1	0.264	Tukey-K	0.872
T3	3	AgeCat*Trt*Glucose	Old Glu p	G4 G5	0.416	0.7	165	0.64	0.525	Tukey-K	0.988
T3	3	AgeCat*Trt*Glucose	Young Glu m	G0 G1	0.209	0.8	165	0.28	0.782	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Young Glu m	G0 G2	1.278	0.8	165	1.7	0.092	Tukey-K	0.537
T3	3	AgeCat*Trt*Glucose	Young Glu m	G0 G3	0.809	0.8	165	1.07	0.285	Tukey-K	0.891
T3	3	AgeCat*Trt*Glucose	Young Glu m	G0 G4	0.745	0.8	165	0.99	0.324	Tukey-K	0.921
T3	3	AgeCat*Trt*Glucose	Young Glu m	G0 G5	1.096	0.8	165	1.45	0.148	Tukey-K	0.694
T3	3	AgeCat*Trt*Glucose	Young Glu m	G1 G2	1.069	0.8	165	1.42	0.158	Tukey-K	0.716
T3	3	AgeCat*Trt*Glucose	Young Glu m	G1 G3	0.6	0.8	165	0.8	0.427	Tukey-K	0.968
T3	3	AgeCat*Trt*Glucose	Young Glu m	G1 G4	0.536	0.8	165	0.71	0.478	Tukey-K	0.98
T3	3	AgeCat*Trt*Glucose	Young Glu m	G1 G5	0.887	0.8	165	1.18	0.241	Tukey-K	0.847
T3	3	AgeCat*Trt*Glucose	Young Glu m	G2 G3	-0.47	0.8	165	-0.6	0.535	Tukey-K	0.989
T3	3	AgeCat*Trt*Glucose	Young Glu m	G2 G4	-0.53	0.8	165	-0.7	0.481	Tukey-K	0.981
T3	3	AgeCat*Trt*Glucose	Young Glu m	G2 G5	-0.18	0.8	165	-0.2	0.81	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Young Glu m	G3 G4	-0.06	0.8	165	-0.1	0.933	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Young Glu m	G3 G5	0.287	0.8	165	0.38	0.704	Tukey-K	0.999
T3	3	AgeCat*Trt*Glucose	Young Glu m	G4 G5	0.351	0.8	165	0.47	0.642	Tukey-K	0.997
T3	3	AgeCat*Trt*Glucose	Young Glu p	G0 G1	0.82	0.8	165	1.09	0.278	Tukey-K	0.885
T3	3	AgeCat*Trt*Glucose	Young Glu p	G0 G2	1.106	0.8	165	1.47	0.144	Tukey-K	0.685
T3	3	AgeCat*Trt*Glucose	Young Glu p	G0 G3	0.67	0.8	165	0.89	0.375	Tukey-K	0.949
T3	3	AgeCat*Trt*Glucose	Young Glu p	G0 G4	0.584	0.8	165	0.78	0.439	Tukey-K	0.971
T3	3	AgeCat*Trt*Glucose	Young Glu p	G0 G5	0.027	0.8	165	0.04	0.972	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Young Glu p	G1 G2	0.286	0.8	165	0.38	0.705	Tukey-K	0.999
T3	3	AgeCat*Trt*Glucose	Young Glu p	G1 G3	-0.15	0.8	165	-0.2	0.842	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Young Glu p	G1 G4	-0.24	0.8	165	-0.3	0.754	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Young Glu p	G1 G5	-0.79	0.8	165	-1.1	0.294	Tukey-K	0.899
T3	3	AgeCat*Trt*Glucose	Young Glu p	G2 G3	-0.44	0.8	165	-0.6	0.563	Tukey-K	0.992
T3	3	AgeCat*Trt*Glucose	Young Glu p	G2 G4	-0.52	0.8	165	-0.7	0.489	Tukey-K	0.983
T3	3	AgeCat*Trt*Glucose	Young Glu p	G2 G5	-1.08	0.8	165	-1.4	0.154	Tukey-K	0.707
T3	3	AgeCat*Trt*Glucose	Young Glu p	G3 G4	-0.09	0.8	165	-0.1	0.909	Tukey-K	1
T3	3	AgeCat*Trt*Glucose	Young Glu p	G3 G5	-0.64	0.8	165	-0.9	0.395	Tukey-K	0.957
T3	3	AgeCat*Trt*Glucose	Young Glu p	G4 G5	-0.56	0.8	165	-0.7	0.461	Tukey-K	0.977
T4	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G1	-0.3	1	165	-0.3	0.759	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G2	0.25	1	165	0.26	0.798	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G3	0.274	1	165	0.28	0.779	Tukey-K	1

T4	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G4	-1.04	1	165	-1.1	0.288	Tukey-K	0.894
T4	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G5	-0.04	1	165	-0	0.965	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G2	0.55	1	165	0.56	0.573	Tukey-K	0.993
T4	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G3	0.574	1	165	0.59	0.556	Tukey-K	0.992
T4	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G4	-0.74	1	165	-0.8	0.449	Tukey-K	0.974
T4	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G5	0.258	1	165	0.26	0.792	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G3	0.024	1	165	0.03	0.98	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G4	-1.29	1	165	-1.3	0.188	Tukey-K	0.772
T4	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G5	-0.29	1	165	-0.3	0.764	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Middle Glu m	G3 G4	-1.31	1	165	-1.3	0.179	Tukey-K	0.757
T4	3	AgeCat*Trt*Glucose	Middle Glu m	G3 G5	-0.32	1	165	-0.3	0.745	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Middle Glu m	G4 G5	0.997	1	165	1.02	0.308	Tukey-K	0.91
T4	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G1	0.869	1	165	0.89	0.374	Tukey-K	0.948
T4	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G2	-0.52	1	165	-0.5	0.592	Tukey-K	0.995
T4	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G3	-0.97	1	165	-1	0.323	Tukey-K	0.92
T4	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G4	0.088	1	165	0.09	0.928	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G5	-1.37	1	165	-1.4	0.162	Tukey-K	0.725
T4	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G2	-1.39	1	165	-1.4	0.155	Tukey-K	0.709
T4	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G3	-1.83	1	165	-1.9	0.061	Tukey-K	0.416
T4	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G4	-0.78	1	165	-0.8	0.424	Tukey-K	0.967
T4	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G5	-2.24	1	165	-2.3	0.023	Tukey-K	0.202
T4	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G3	-0.44	1	165	-0.5	0.65	Tukey-K	0.998
T4	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G4	0.611	1	165	0.63	0.532	Tukey-K	0.989
T4	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G5	-0.84	1	165	-0.9	0.388	Tukey-K	0.954
T4	3	AgeCat*Trt*Glucose	Middle Glu p	G3 G4	1.053	1	165	1.08	0.281	Tukey-K	0.888
T4	3	AgeCat*Trt*Glucose	Middle Glu p	G3 G5	-0.4	1	165	-0.4	0.681	Tukey-K	0.998
T4	3	AgeCat*Trt*Glucose	Middle Glu p	G4 G5	-1.45	1	165	-1.5	0.137	Tukey-K	0.669
T4	3	AgeCat*Trt*Glucose	Old Glu m	G0 G1	0.302	0.7	165	0.44	0.661	Tukey-K	0.998
T4	3	AgeCat*Trt*Glucose	Old Glu m	G0 G2	-0.74	0.7	165	-1.1	0.283	Tukey-K	0.89
T4	3	AgeCat*Trt*Glucose	Old Glu m	G0 G3	-0.3	0.7	165	-0.4	0.663	Tukey-K	0.998
T4	3	AgeCat*Trt*Glucose	Old Glu m	G0 G4	-0.03	0.7	165	-0	0.968	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Old Glu m	G0 G5	0.166	0.7	165	0.24	0.81	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Old Glu m	G1 G2	-1.04	0.7	165	-1.5	0.132	Tukey-K	0.655
T4	3	AgeCat*Trt*Glucose	Old Glu m	G1 G3	-0.6	0.7	165	-0.9	0.383	Tukey-K	0.952
T4	3	AgeCat*Trt*Glucose	Old Glu m	G1 G4	-0.33	0.7	165	-0.5	0.633	Tukey-K	0.997
T4	3	AgeCat*Trt*Glucose	Old Glu m	G1 G5	-0.14	0.7	165	-0.2	0.843	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Old Glu m	G2 G3	0.441	0.7	165	0.64	0.523	Tukey-K	0.988
T4	3	AgeCat*Trt*Glucose	Old Glu m	G2 G4	0.714	0.7	165	1.04	0.301	Tukey-K	0.905
T4	3	AgeCat*Trt*Glucose	Old Glu m	G2 G5	0.908	0.7	165	1.32	0.19	Tukey-K	0.775
T4	3	AgeCat*Trt*Glucose	Old Glu m	G3 G4	0.273	0.7	165	0.4	0.692	Tukey-K	0.999
T4	3	AgeCat*Trt*Glucose	Old Glu m	G3 G5	0.467	0.7	165	0.68	0.499	Tukey-K	0.984

T4	3	AgeCat*Trt*Glucose	Old Glu m	G4 G5	0.193	0.7	165	0.28	0.779	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Old Glu p	G0 G1	-0.78	0.7	165	-1.1	0.258	Tukey-K	0.866
T4	3	AgeCat*Trt*Glucose	Old Glu p	G0 G2	-0.47	0.7	165	-0.7	0.495	Tukey-K	0.983
T4	3	AgeCat*Trt*Glucose	Old Glu p	G0 G3	0.006	0.7	165	0.01	0.993	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Old Glu p	G0 G4	-0.35	0.7	165	-0.5	0.61	Tukey-K	0.996
T4	3	AgeCat*Trt*Glucose	Old Glu p	G0 G5	0.091	0.7	165	0.13	0.895	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Old Glu p	G1 G2	0.31	0.7	165	0.45	0.653	Tukey-K	0.998
T4	3	AgeCat*Trt*Glucose	Old Glu p	G1 G3	0.788	0.7	165	1.14	0.255	Tukey-K	0.863
T4	3	AgeCat*Trt*Glucose	Old Glu p	G1 G4	0.429	0.7	165	0.62	0.534	Tukey-K	0.989
T4	3	AgeCat*Trt*Glucose	Old Glu p	G1 G5	0.873	0.7	165	1.27	0.207	Tukey-K	0.803
T4	3	AgeCat*Trt*Glucose	Old Glu p	G2 G3	0.478	0.7	165	0.69	0.489	Tukey-K	0.983
T4	3	AgeCat*Trt*Glucose	Old Glu p	G2 G4	0.119	0.7	165	0.17	0.863	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Old Glu p	G2 G5	0.563	0.7	165	0.82	0.415	Tukey-K r	0.964
T4	3	AgeCat*Trt*Glucose	Old Glu p	G3 G4	-0.36	0.7	165	-0.5	0.604	Tukey-K	0.995
T4	3	AgeCat*Trt*Glucose	Old Glu p	G3 G5	0.085	0.7	165	0.12	0.902	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Old Glu p	G4 G5	0.443	0.7	165	0.64	0.521	Tukey-K	0.987
T4	3	AgeCat*Trt*Glucose	Young Glu m	G0 G1	0.194	0.8	165	0.24	0.808	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Young Glu m	G0 G2	0.028	0.8	165	0.03	0.972	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Young Glu m	G0 G3	-0.14	0.8	165	-0.2	0.864	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Young Glu m	G0 G4	0.537	0.8	165	0.67	0.501	Tukey-K	0.985
T4	3	AgeCat*Trt*Glucose	Young Glu m	G0 G5	0.539	0.8	165	0.68	0.499	Tukey-K	0.984
T4	3	AgeCat*Trt*Glucose	Young Glu m	G1 G2	-0.17	0.8	165	-0.2	0.835	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Young Glu m	G1 G3	-0.33	0.8	165	-0.4	0.679	Tukey-K	0.998
T4	3	AgeCat*Trt*Glucose	Young Glu m	G1 G4	0.343	0.8	165	0.43	0.667	Tukey-K	0.998
T4	3	AgeCat*Trt*Glucose	Young Glu m	G1 G5	0.345	0.8	165	0.43	0.665	Tukey-K	0.998
T4	3	AgeCat*Trt*Glucose	Young Glu m	G2 G3	-0.16	0.8	165	-0.2	0.837	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Young Glu m	G2 G4	0.509	0.8	165	0.64	0.523	Tukey-K	0.988
T4	3	AgeCat*Trt*Glucose	Young Glu m	G2 G5	0.512	0.8	165	0.64	0.521	Tukey-K	0.988
T4	3	AgeCat*Trt*Glucose	Young Glu m	G3 G4	0.673	0.8	165	0.85	0.399	Tukey-K	0.958
T4	3	AgeCat*Trt*Glucose	Young Glu m	G3 G5	0.675	0.8	165	0.85	0.397	Tukey-K	0.958
T4	3	AgeCat*Trt*Glucose	Young Glu m	G4 G5	0.003	0.8	165	0	0.997	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Young Glu p	G0 G1	0.596	0.8	165	0.75	0.455	Tukey-K	0.975
T4	3	AgeCat*Trt*Glucose	Young Glu p	G0 G2	0.747	0.8	165	0.94	0.349	Tukey-K	0.936
T4	3	AgeCat*Trt*Glucose	Young Glu p	G0 G3	0.704	0.8	165	0.89	0.377	Tukey-K	0.95
T4	3	AgeCat*Trt*Glucose	Young Glu p	G0 G4	0.83	0.8	165	1.04	0.298	Tukey-K	0.902
T4	3	AgeCat*Trt*Glucose	Young Glu p	G0 G5	0.999	0.8	165	1.26	0.211	Tukey-K	0.808
T4	3	AgeCat*Trt*Glucose	Young Glu p	G1 G2	0.151	0.8	165	0.19	0.85	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Young Glu p	G1 G3	0.108	0.8	165	0.14	0.892	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Young Glu p	G1 G4	0.234	0.8	165	0.29	0.769	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Young Glu p	G1 G5	0.403	0.8	165	0.51	0.613	Tukey-K	0.996
T4	3	AgeCat*Trt*Glucose	Young Glu p	G2 G3	-0.04	0.8	165	-0.1	0.957	Tukey-K	1

T4	3	AgeCat*Trt*Glucose	Young Glu p	G2 G4	0.083	0.8	165	0.1	0.917	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Young Glu p	G2 G5	0.252	0.8	165	0.32	0.752	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Young Glu p	G3 G4	0.126	0.8	165	0.16	0.875	Tukey-K	1
T4	3	AgeCat*Trt*Glucose	Young Glu p	G3 G5	0.295	0.8	165	0.37	0.711	Tukey-K	0.999
T4	3	AgeCat*Trt*Glucose	Young Glu p	G4 G5	0.169	0.8	165	0.21	0.832	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G1	1.483	1	165	1.43	0.156	Tukey-K	0.711
T5	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G2	1.859	1	165	1.79	0.076	Tukey-K	0.476
T5	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G3	1.642	1	165	1.58	0.116	Tukey-K	0.613
T5	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G4	1.208	1	165	1.16	0.247	Tukey-K	0.854
T5	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G5	1.482	1	165	1.43	0.156	Tukey-K	0.711
T5	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G2	0.376	1	165	0.36	0.718	Tukey-K	0.999
T5	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G3	0.159	1	165	0.15	0.878	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G4	-0.27	1	165	-0.3	0.792	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G5	-0	1	165	-0	1	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G3	-0.22	1	165	-0.2	0.835	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G4	-0.65	1	165	-0.6	0.532	Tukey-K	0.989
T5	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G5	-0.38	1	165	-0.4	0.718	Tukey-K	0.999
T5	3	AgeCat*Trt*Glucose	Middle Glu m	G3 G4	-0.43	1	165	-0.4	0.677	Tukey-K	0.998
T5	3	AgeCat*Trt*Glucose	Middle Glu m	G3 G5	-0.16	1	165	-0.2	0.878	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Middle Glu m	G4 G5	0.274	1	165	0.26	0.792	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G1	1.125	1	165	1.08	0.28	Tukey-K	0.888
T5	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G2	1.217	1	165	1.17	0.243	Tukey-K	0.85
T5	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G3	-0.04	1	165	-0	0.971	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G4	1.14	1	165	1.1	0.274	Tukey-K	0.882
T5	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G5	-0.36	1	165	-0.3	0.733	Tukey-K	0.999
T5	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G2	0.091	1	165	0.09	0.93	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G3	-1.16	1	165	-1.1	0.265	Tukey-K	0.873
T5	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G4	0.015	1	165	0.01	0.989	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G5	-1.48	1	165	-1.4	0.156	Tukey-K	0.712
T5	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G3	-1.26	1	165	-1.2	0.229	Tukey-K	0.833
T5	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G4	-0.08	1	165	-0.1	0.941	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G5	-1.57	1	165	-1.5	0.132	Tukey-K	0.656
T5	3	AgeCat*Trt*Glucose	Middle Glu p	G3 G4	1.178	1	165	1.13	0.259	Tukey-K	0.867
T5	3	AgeCat*Trt*Glucose	Middle Glu p	G3 G5	-0.32	1	165	-0.3	0.76	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Middle Glu p	G4 G5	-1.5	1	165	-1.4	0.152	Tukey-K	0.703
T5	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G1	1.293	0.7	165	1.76	0.08	Tukey-K	0.494
T5	3	AgeCat*Trt*Glucose	Old Glu m	G0 G2	1.06	0.7	165	1.44	0.151	Tukey-K	0.701
T5	3	AgeCat*Trt*Glucose	Old Glu m	G0 G3	0.673	0.7	165	0.92	0.361	Tukey-K	0.942
T5	3	AgeCat*Trt*Glucose	Old Glu m	G0 G4	0.532	0.7	165	0.72	0.47	Tukey-K	0.979
T5	3	AgeCat*Trt*Glucose	Old Glu m	G0 G5	1.005	0.7	165	1.37	0.173	Tukey-K	0.747
T5	3	AgeCat*Trt*Glucose	Old Glu m	G1 G2	-0.23	0.7	165	-0.3	0.751	Tukey-K	1

T5	3	AgeCat*Trt*Glucose	Old Glu m	G1 G3	-0.62	0.7	165	-0.8	0.4	Tukey-K	0.959
T5	3	AgeCat*Trt*Glucose	Old Glu m	G1 G4	-0.76	0.7	165	-1	0.302	Tukey-K	0.905
T5	3	AgeCat*Trt*Glucose	Old Glu m	G1 G5	-0.29	0.7	165	-0.4	0.695	Tukey-K	0.999
T5	3	AgeCat*Trt*Glucose	Old Glu m	G2 G3	-0.39	0.7	165	-0.5	0.599	Tukey-K	0.995
T5	3	AgeCat*Trt*Glucose	Old Glu m	G2 G4	-0.53	0.7	165	-0.7	0.474	Tukey-K	0.98
T5	3	AgeCat*Trt*Glucose	Old Glu m	G2 G5	-0.06	0.7	165	-0.1	0.94	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Old Glu m	G3 G4	-0.14	0.7	165	-0.2	0.849	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Old Glu m	G3 G5	0.332	0.7	165	0.45	0.652	Tukey-K	0.998
T5	3	AgeCat*Trt*Glucose	Old Glu m	G4 G5	0.472	0.7	165	0.64	0.521	Tukey-K	0.988
T5	3	AgeCat*Trt*Glucose	Old Glu p	G0 G1	0.028	0.7	165	0.04	0.97	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Old Glu p	G0 G2	1.213	0.7	165	1.65	0.101	Tukey-K	0.566
T5	3	AgeCat*Trt*Glucose	Old Glu p	G0 G3	0.748	0.7	165	1.02	0.31	Tukey-K	0.911
T5	3	AgeCat*Trt*Glucose	Old Glu p	G0 G4	-0.01	0.7	165	-0	0.991	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Old Glu p	G0 G5	0.428	0.7	165	0.58	0.561	Tukey-K	0.992
T5	3	AgeCat*Trt*Glucose	Old Glu p	G1 G2	1.186	0.7	165	1.61	0.109	Tukey-K	0.591
T5	3	AgeCat*Trt*Glucose	Old Glu p	G1 G3	0.721	0.7	165	0.98	0.328	Tukey-K	0.924
T5	3	AgeCat*Trt*Glucose	Old Glu p	G1 G4	-0.04	0.7	165	-0	0.961	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Old Glu p	G1 G5	0.401	0.7	165	0.55	0.586	Tukey-K	0.994
T5	3	AgeCat*Trt*Glucose	Old Glu p	G2 G3	-0.46	0.7	165	-0.6	0.528	Tukey-K	0.988
T5	3	AgeCat*Trt*Glucose	Old Glu p	G2 G4	-1.22	0.7	165	-1.7	0.098	Tukey-K	0.559
T5	3	AgeCat*Trt*Glucose	Old Glu p	G2 G5	-0.79	0.7	165	-1.1	0.287	Tukey-K	0.893
T5	3	AgeCat*Trt*Glucose	Old Glu p	G3 G4	-0.76	0.7	165	-1	0.305	Tukey-K	0.907
T5	3	AgeCat*Trt*Glucose	Old Glu p	G3 G5	-0.32	0.7	165	-0.4	0.664	Tukey-K	0.998
T5	3	AgeCat*Trt*Glucose	Old Glu p	G4 G5	0.437	0.7	165	0.59	0.553	Tukey-K	0.991
T5	3	AgeCat*Trt*Glucose	Young Glu m	G0 G1	-0.26	0.8	165	-0.3	0.757	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Young Glu m	G0 G2	-0.95	0.8	165	-1.1	0.267	Tukey-K	0.875
T5	3	AgeCat*Trt*Glucose	Young Glu m	G0 G3	-0.98	0.8	165	-1.2	0.249	Tukey-K	0.856
T5	3	AgeCat*Trt*Glucose	Young Glu m	G0 G4	-0.19	0.8	165	-0.2	0.822	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Young Glu m	G0 G5	0.325	0.8	165	0.38	0.702	Tukey-K	0.999
T5	3	AgeCat*Trt*Glucose	Young Glu m	G1 G2	-0.68	0.8	165	-0.8	0.423	Tukey-K	0.966
T5	3	AgeCat*Trt*Glucose	Young Glu m	G1 G3	-0.72	0.8	165	-0.8	0.398	Tukey-K	0.958
T5	3	AgeCat*Trt*Glucose	Young Glu m	G1 G4	0.072	0.8	165	0.08	0.932	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Young Glu m	G1 G5	0.588	0.8	165	0.69	0.489	Tukey-K	0.983
T5	3	AgeCat*Trt*Glucose	Young Glu m	G2 G3	-0.04	0.8	165	-0	0.965	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Young Glu m	G2 G4	0.754	0.8	165	0.89	0.375	Tukey-K	0.949
T5	3	AgeCat*Trt*Glucose	Young Glu m	G2 G5	1.27	0.8	165	1.5	0.136	Tukey-K	0.667
T5	3	AgeCat*Trt*Glucose	Young Glu m	G3 G4	0.791	0.8	165	0.93	0.352	Tukey-K	0.938
T5	3	AgeCat*Trt*Glucose	Young Glu m	G3 G5	1.307	0.8	165	1.54	0.125	Tukey-K	0.639
T5	3	AgeCat*Trt*Glucose	Young Glu m	G4 G5	0.516	0.8	165	0.61	0.544	Tukey-K	0.99
T5	3	AgeCat*Trt*Glucose	Young Glu p	G0 G1	-0.44	0.8	165	-0.5	0.604	Tukey-K	0.995
T5	3	AgeCat*Trt*Glucose	Young Glu p	G0 G2	-1.12	0.8	165	-1.3	0.189	Tukey-K	0.774

T5	3	AgeCat*Trt*Glucose	Young Glu p	G0 G3	-0.82	0.8	165	-1	0.334	Tukey-K	0.927
T5	3	AgeCat*Trt*Glucose	Young Glu p	G0 G4	-1.29	0.8	165	-1.5	0.13	Tukey-K	0.652
T5	3	AgeCat*Trt*Glucose	Young Glu p	G0 G5	-0.62	0.8	165	-0.7	0.464	Tukey-K	0.977
T5	3	AgeCat*Trt*Glucose	Young Glu p	G1 G2	-0.68	0.8	165	-0.8	0.425	Tukey-K	0.967
T5	3	AgeCat*Trt*Glucose	Young Glu p	G1 G3	-0.38	0.8	165	-0.4	0.655	Tukey-K	0.998
T5	3	AgeCat*Trt*Glucose	Young Glu p	G1 G4	-0.85	0.8	165	-1	0.319	Tukey-K	0.917
T5	3	AgeCat*Trt*Glucose	Young Glu p	G1 G5	-0.18	0.8	165	-0.2	0.83	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Young Glu p	G2 G3	0.298	0.8	165	0.35	0.726	Tukey-K	0.999
T5	3	AgeCat*Trt*Glucose	Young Glu p	G2 G4	-0.17	0.8	165	-0.2	0.841	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Young Glu p	G2 G5	0.496	0.8	165	0.58	0.559	Tukey-K	0.992
T5	3	AgeCat*Trt*Glucose	Young Glu p	G3 G4	-0.47	0.8	165	-0.6	0.582	Tukey-K	0.994
T5	3	AgeCat*Trt*Glucose	Young Glu p	G3 G5	0.198	0.8	165	0.23	0.815	Tukey-K	1
T5	3	AgeCat*Trt*Glucose	Young Glu p	G4 G5	0.667	0.8	165	0.79	0.433	Tukey-K	0.97
T6	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G1	-0.42	1.6	165	-0.3	0.793	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G2	0.231	1.6	165	0.14	0.886	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G3	2.383	1.6	165	1.48	0.14	Tukey-K	0.677
T6	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G4	0.788	1.6	165	0.49	0.625	Tukey-K	0.996
T6	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G5	2.524	1.6	165	1.57	0.119	Tukey-K	0.62
T6	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G2	0.655	1.6	165	0.41	0.684	Tukey-K	0.999
T6	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G3	2.807	1.6	165	1.74	0.083	Tukey-K	0.504
T6	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G4	1.212	1.6	165	0.75	0.452	Tukey-K	0.975
T6	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G5	2.947	1.6	165	1.83	0.069	Tukey-K	0.448
T6	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G3	2.152	1.6	165	1.34	0.183	Tukey-K	0.764
T6	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G4	0.557	1.6	165	0.35	0.73	Tukey-K	0.999
T6	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G5	2.292	1.6	165	1.42	0.156	Tukey-K	0.712
T6	3	AgeCat*Trt*Glucose	Middle Glu m	G3 G4	-1.6	1.6	165	-1	0.323	Tukey-K	0.92
T6	3	AgeCat*Trt*Glucose	Middle Glu m	G3 G5	0.141	1.6	165	0.09	0.93	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Middle Glu m	G4 G5	1.736	1.6	165	1.08	0.282	Tukey-K	0.889
T6	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G1	2.054	1.6	165	1.28	0.203	Tukey-K	0.797
T6	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G2	0.803	1.6	165	0.5	0.618	Tukey-K	0.996
T6	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G3	1.046	1.6	165	0.65	0.517	Tukey-K	0.987
T6	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G4	2.776	1.6	165	1.73	0.086	Tukey-K	0.517
T6	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G5	0.573	1.6	165	0.36	0.722	Tukey-K	0.999
T6	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G2	-1.25	1.6	165	-0.8	0.438	Tukey-K	0.971
T6	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G3	-1.01	1.6	165	-0.6	0.532	Tukey-K	0.989
T6	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G4	0.722	1.6	165	0.45	0.654	Tukey-K	0.998
T6	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G5	-1.48	1.6	165	-0.9	0.359	Tukey-K	0.941
T6	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G3	0.242	1.6	165	0.15	0.88	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G4	1.973	1.6	165	1.23	0.222	Tukey-K	0.823
T6	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G5	-0.23	1.6	165	-0.1	0.886	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Middle Glu p	G3 G4	1.731	1.6	165	1.08	0.284	Tukey-K	0.89

T6	3	AgeCat*Trt*Glucose	Middle Glu p	G3 G5	-0.47	1.6	165	-0.3	0.769	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Middle Glu p	G4 G5	-2.2	1.6	165	-1.4	0.173	Tukey-K	0.745
T6	3	AgeCat*Trt*Glucose	Old Glu m	G0 G1	0.508	1.1	165	0.45	0.656	Tukey-K	0.998
T6	3	AgeCat*Trt*Glucose	Old Glu m	G0 G2	0.914	1.1	165	0.8	0.423	Tukey-K	0.967
T6	3	AgeCat*Trt*Glucose	OldGlu m	G0 G3	0.626	1.1	165	0.55	0.583	Tukey-K	0.994
T6	3	AgeCat*Trt*Glucose	Old Glu m	G0 G4	-0.19	1.1	165	-0.2	0.868	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Old Glu m	G0 G5	0.867	1.1	165	0.76	0.447	Tukey-K	0.973
T6	3	AgeCat*Trt*Glucose	Old Glu m	G1 G2	0.406	1.1	165	0.36	0.721	Tukey-K	0.999
T6	3	AgeCat*Trt*Glucose	Old Glu m	G1 G3	0.118	1.1	165	0.1	0.917	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Old Glu m	G1 G4	-0.7	1.1	165	-0.6	0.541	Tukey-K	0.99
T6	3	AgeCat*Trt*Glucose	Old Glu m	G1 G5	0.359	1.1	165	0.32	0.753	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Old Glu m	G2 G3	-0.29	1.1	165	-0.3	0.8	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Old Glu m	G2 G4	-1.1	1.1	165	-1	0.333	Tukey-K	0.927
T6	3	AgeCat*Trt*Glucose	Old Glu m	G2 G5	-0.05	1.1	165	-0	0.967	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Old Glu m	G3 G4	-0.82	1.1	165	-0.7	0.475	Tukey-K	0.98
T6	3	AgeCat*Trt*Glucose	Old Glu m	G3 G5	0.241	1.1	165	0.21	0.833	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Old Glu m	G4 G5	1.056	1.1	165	0.93	0.355	Tukey-K	0.939
T6	3	AgeCat*Trt*Glucose	Old Glu p	G0 G1	0.346	1.1	165	0.3	0.761	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Old Glu p	G0 G2	1.348	1.1	165	1.19	0.238	Tukey-K	0.843
T6	3	AgeCat*Trt*Glucose	Old Glu p	G0 G3	2.207	1.1	165	1.94	0.054	Tukey-K	0.382
T6	3	AgeCat*Trt*Glucose	Old Glu p	G0 G4	0.944	1.1	165	0.83	0.408	Tukey-K	0.962
T6	3	AgeCat*Trt*Glucose	Old Glu p	G0 G5	1.771	1.1	165	1.56	0.121	Tukey-K	0.628
T6	3	AgeCat*Trt*Glucose	Old Glu p	G1 G2	1.003	1.1	165	0.88	0.379	Tukey-K	0.951
T6	3	AgeCat*Trt*Glucose	Old Glu p	G1 G3	1.861	1.1	165	1.64	0.104	Tukey-K	0.576
T6	3	AgeCat*Trt*Glucose	Old Glu p	G1 G4	0.598	1.1	165	0.53	0.6	Tukey-K	0.995
T6	3	AgeCat*Trt*Glucose	Old Glu p	G1 G5	1.425	1.1	165	1.25	0.212	Tukey-K	0.81
T6	3	AgeCat*Trt*Glucose	Old Glu p	G2 G3	0.859	1.1	165	0.75	0.451	Tukey-K	0.974
T6	3	AgeCat*Trt*Glucose	Old Glu p	G2 G4	-0.4	1.1	165	-0.4	0.723	Tukey-K	0.999
T6	3	AgeCat*Trt*Glucose	Old Glu p	G2 G5	0.422	1.1	165	0.37	0.711	Tukey-K	0.999
T6	3	AgeCat*Trt*Glucose	Old Glu p	G3 G4	-1.26	1.1	165	-1.1	0.269	Tukey-K	0.877
T6	3	AgeCat*Trt*Glucose	Old Glu p	G3 G5	-0.44	1.1	165	-0.4	0.702	Tukey-K	0.999
T6	3	AgeCat*Trt*Glucose	Old Glu p	G4 G5	0.827	1.1	165	0.73	0.469	Tukey-K	0.978
T6	3	AgeCat*Trt*Glucose	Young Glu m	G0 G1	1.525	1.3	165	1.16	0.247	Tukey-K	0.855
T6	3	AgeCat*Trt*Glucose	Young Glu m	G0 G2	2.334	1.3	165	1.78	0.077	Tukey-K	0.483
T6	3	AgeCat*Trt*Glucose	Young Glu m	G0 G3	0.748	1.3	165	0.57	0.57	Tukey-K	0.993
T6	3	AgeCat*Trt*Glucose	Young Glu m	G0 G4	2.135	1.3	165	1.63	0.106	Tukey-K	0.583
T6	3	AgeCat*Trt*Glucose	Young Glu m	G0 G5	0.677	1.3	165	0.52	0.607	Tukey-K	0.996
T6	3	AgeCat*Trt*Glucose	Young Glu m	G1 G2	0.81	1.3	165	0.62	0.539	Tukey-K	0.99
T6	3	AgeCat*Trt*Glucose	Young Glu m	G1 G3	-0.78	1.3	165	-0.6	0.555	Tukey-K	0.992
T6	3	AgeCat*Trt*Glucose	Young Glu m	G1 G4	0.61	1.3	165	0.46	0.643	Tukey-K	0.997
T6	3	AgeCat*Trt*Glucose	Young Glu m	G1 G5	-0.85	1.3	165	-0.6	0.52	Tukey-K	0.987

T6	3	AgeCat*Trt*Glucose	Young Glu m	G2 G3	-1.59	1.3	165	-1.2	0.229	Tukey-K	0.833
T6	3	AgeCat*Trt*Glucose	Young Glu m	G2 G4	-0.2	1.3	165	-0.2	0.88	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Young Glu m	G2 G5	-1.66	1.3	165	-1.3	0.209	Tukey-K	0.805
T6	3	AgeCat*Trt*Glucose	Young Glu m	G3 G4	1.387	1.3	165	1.06	0.293	Tukey-K	0.898
T6	3	AgeCat*Trt*Glucose	Young Glu m	G3 G5	-0.07	1.3	165	-0.1	0.957	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Young Glu m	G4 G5	-1.46	1.3	165	-1.1	0.269	Tukey-K	0.877
T6	3	AgeCat*Trt*Glucose	Young Glu p	G0 G1	0.168	1.3	165	0.13	0.898	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Young Glu p	G0 G2	1.591	1.3	165	1.21	0.227	Tukey-K	0.831
T6	3	AgeCat*Trt*Glucose	Young Glu p	G0 G3	0.329	1.3	165	0.25	0.802	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Young Glu p	G0 G4	0.538	1.3	165	0.41	0.683	Tukey-K	0.999
T6	3	AgeCat*Trt*Glucose	Young Glu p	G0 G5	0.142	1.3	165	0.11	0.914	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Young Glu p	G1 G2	1.423	1.3	165	1.08	0.28	Tukey-K	0.887
T6	3	AgeCat*Trt*Glucose	Young Glu p	G1 G3	0.161	1.3	165	0.12	0.902	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Young Glu p	G1 G4	0.37	1.3	165	0.28	0.779	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Young Glu p	G1 G5	-0.03	1.3	165	-0	0.984	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Young Glu p	G2 G3	-1.26	1.3	165	-1	0.338	Tukey-K	0.93
T6	3	AgeCat*Trt*Glucose	Young Glu p	G2 G4	-1.05	1.3	165	-0.8	0.424	Tukey-K	0.967
T6	3	AgeCat*Trt*Glucose	Young Glu p	G2 G5	-1.45	1.3	165	-1.1	0.271	Tukey-K	0.879
T6	3	AgeCat*Trt*Glucose	Young Glu p	G3 G4	0.208	1.3	165	0.16	0.874	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Young Glu p	G3 G5	-0.19	1.3	165	-0.1	0.887	Tukey-K	1
T6	3	AgeCat*Trt*Glucose	Young Glu p	G4 G5	-0.4	1.3	165	-0.3	0.764	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G1	0.242	1.4	165	0.18	0.86	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G2	0.92	1.4	165	0.67	0.502	Tukey-K	0.985
T7	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G3	0.897	1.4	165	0.66	0.513	Tukey-Kramer	0.986
T7	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G4	0.024	1.4	165	0.02	0.986	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G5	1.177	1.4	165	0.86	0.391	Tukey-K	0.955
T7	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G2	0.678	1.4	165	0.5	0.621	Tukey-K	0.996
T7	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G3	0.654	1.4	165	0.48	0.633	Tukey-K	0.997
T7	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G4	-0.22	1.4	165	-0.2	0.873	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G5	0.935	1.4	165	0.68	0.495	Tukey-K	0.984
T7	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G3	-0.02	1.4	165	-0	0.987	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G4	-0.9	1.4	165	-0.7	0.513	Tukey-K	0.986
T7	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G5	0.257	1.4	165	0.19	0.851	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Middle Glu m	G3 G4	-0.87	1.4	165	-0.6	0.524	Tukey-K	0.988
T7	3	AgeCat*Trt*Glucose	Middle Glu m	G3 G5	0.281	1.4	165	0.21	0.838	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Middle Glu m	G4 G5	1.153	1.4	165	0.84	0.4	Tukey-K	0.959
T7	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G1	1.164	1.4	165	0.85	0.396	Tukey-K	0.957
T7	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G2	-0.56	1.4	165	-0.4	0.684	Tukey-K	0.999
T7	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G3	1.141	1.4	165	0.83	0.405	Tukey-K	0.961
T7	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G4	1.416	1.4	165	1.04	0.302	Tukey-K	0.905
T7	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G5	0.602	1.4	165	0.44	0.66	Tukey-K	0.998

T7	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G2	-1.72	1.4	165	-1.3	0.21	Tukey-K	0.807
T7	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G3	-0.02	1.4	165	-0	0.986	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G4	0.251	1.4	165	0.18	0.854	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G5	-0.56	1.4	165	-0.4	0.681	Tukey-K	0.998
T7	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G3	1.698	1.4	165	1.24	0.216	Tukey-K	0.816
T7	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G4	1.972	1.4	165	1.44	0.151	Tukey-K	0.701
T7	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G5	1.159	1.4	165	0.85	0.398	Tukey-K	0.958
T7	3	AgeCat*Trt*Glucose	Middle Glu p	G3 G4	0.275	1.4	165	0.2	0.841	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Middle Glu p	G3 G5	-0.54	1.4	165	-0.4	0.694	Tukey-K	0.999
T7	3	AgeCat*Trt*Glucose	Middle Glu p	G4 G5	-0.81	1.4	165	-0.6	0.553	Tukey-K	0.991
T7	3	AgeCat*Trt*Glucose	Old Glu m	G0 G1	1.096	1	165	1.13	0.259	Tukey-K	0.867
T7	3	AgeCat*Trt*Glucose	Old Glu m	G0 G2	0.037	1	165	0.04	0.97	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Old Glu m	G0 G3	-2	1	165	-2.1	0.04	Tukey-K	0.307
T7	3	AgeCat*Trt*Glucose	Old Glu m	G0 G4	-1.01	1	165	-1	0.298	Tukey-K	0.902
T7	3	AgeCat*Trt*Glucose	Old Glu m	G0 G5	0.64	1	165	0.66	0.509	Tukey-K	0.986
T7	3	AgeCat*Trt*Glucose	Old Glu m	G1 G2	-1.06	1	165	-1.1	0.275	Tukey-K	0.883
T7	3	AgeCat*Trt*Glucose	Old Glu m	G1 G3	-3.1	1	165	-3.2	0.002	Tukey-K	0.02
T7	3	AgeCat*Trt*Glucose	Old Glu m	G1 G4	-2.11	1	165	-2.2	0.031	Tukey-K	0.254
T7	3	AgeCat*Trt*Glucose	Old Glu m	G1 G5	-0.46	1	165	-0.5	0.638	Tukey-K	0.997
T7	3	AgeCat*Trt*Glucose	Old Glu m	G2 G3	-2.04	1	165	-2.1	0.036	Tukey-K	0.287
T7	3	AgeCat*Trt*Glucose	Old Glu m	G2 G4	-1.05	1	165	-1.1	0.281	Tukey-K	0.888
T7	3	AgeCat*Trt*Glucose	Old Glu m	G2 G5	0.604	1	165	0.62	0.533	Tukey-K	0.989
T7	3	AgeCat*Trt*Glucose	Old Glu m	G3 G4	0.995	1	165	1.03	0.305	Tukey-K	0.908
T7	3	AgeCat*Trt*Glucose	Old Glu m	G3 G5	2.644	1	165	2.73	0.007	Tukey-K	0.074
T7	3	AgeCat*Trt*Glucose	Old Glu m	G4 G5	1.65	1	165	1.71	0.09	Tukey-K	0.53
T7	3	AgeCat*Trt*Glucose	Old Glu p	G0 G1	0.455	1	165	0.47	0.639	Tukey-K	0.997
T7	3	AgeCat*Trt*Glucose	Old Glu p	G0 G2	2.246	1	165	2.32	0.021	Tukey-K	0.191
T7	3	AgeCat*Trt*Glucose	Old Glu p	G0 G3	0.273	1	165	0.28	0.778	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Old Glu p	G0 G4	-0.34	1	165	-0.4	0.724	Tukey-K	0.999
T7	3	AgeCat*Trt*Glucose	Old Glu p	G0 G5	0.873	1	165	0.9	0.368	Tukey-K	0.945
T7	3	AgeCat*Trt*Glucose	Old Glu p	G1 G2	1.791	1	165	1.85	0.066	Tukey-K	0.435
T7	3	AgeCat*Trt*Glucose	Old Glu p	G1 G3	-0.18	1	165	-0.2	0.852	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Old Glu p	G1 G4	-0.8	1	165	-0.8	0.411	Tukey-K	0.963
T7	3	AgeCat*Trt*Glucose	Old Glu p	G1 G5	0.419	1	165	0.43	0.666	Tukey-K	0.998
T7	3	AgeCat*Trt*Glucose	Old Glu p	G2 G3	-1.97	1	165	-2	0.043	Tukey-K	0.325
T7	3	AgeCat*Trt*Glucose	Old Glu p	G2 G4	-2.59	1	165	-2.7	0.008	Tukey-K	0.086
T7	3	AgeCat*Trt*Glucose	Old Glu p	G2 G5	-1.37	1	165	-1.4	0.158	Tukey-K	0.715
T7	3	AgeCat*Trt*Glucose	Old Glu p	G3 G4	-0.62	1	165	-0.6	0.525	Tukey-K	0.988
T7	3	AgeCat*Trt*Glucose	Old Glu p	G3 G5	0.6	1	165	0.62	0.536	Tukey-K	0.989
T7	3	AgeCat*Trt*Glucose	Old Glu p	G4 G5	1.216	1	165	1.26	0.21	Tukey-K	0.808
T7	3	AgeCat*Trt*Glucose	Young Glu m	G0 G1	2.058	1.1	165	1.84	0.067	Tukey-K	0.441

T7	3	AgeCat*Trt*Glucose	Young Glu m	G0 G2	1.401	1.1	165	1.25	0.211	Tukey-K	0.809
T7	3	AgeCat*Trt*Glucose	Young Glu m	G0 G3	0.519	1.1	165	0.46	0.643	Tukey-K	0.997
T7	3	AgeCat*Trt*Glucose	Young Glu m	G0 G4	0.107	1.1	165	0.1	0.924	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Young Glu m	G0 G5	1.86	1.1	165	1.67	0.098	Tukey-K	0.556
T7	3	AgeCat*Trt*Glucose	Young Glu m	G1 G2	-0.66	1.1	165	-0.6	0.557	Tukey-K	0.992
T7	3	AgeCat*Trt*Glucose	Young Glu m	G1 G3	-1.54	1.1	165	-1.4	0.17	Tukey-K	0.74
T7	3	AgeCat*Trt*Glucose	Young Glu m	G1 G4	-1.95	1.1	165	-1.7	0.082	Tukey-K	0.503
T7	3	AgeCat*Trt*Glucose	Young Glu m	G1 G5	-0.2	1.1	165	-0.2	0.86	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Young Glu m	G2 G3	-0.88	1.1	165	-0.8	0.431	Tukey-K	0.969
T7	3	AgeCat*Trt*Glucose	Young Glu m	G2 G4	-1.29	1.1	165	-1.2	0.248	Tukey-K	0.856
T7	3	AgeCat*Trt*Glucose	Young Glu m	G2 G5	0.459	1.1	165	0.41	0.681	Tukey-K	0.998
T7	3	AgeCat*Trt*Glucose	Young Glu m	G3 G4	-0.41	1.1	165	-0.4	0.713	Tukey-K	0.999
T7	3	AgeCat*Trt*Glucose	Young Glu m	G3 G5	1.341	1.1	165	1.2	0.231	Tukey-K	0.836
T7	3	AgeCat*Trt*Glucose	Young Glu m	G4 G5	1.753	1.1	165	1.57	0.118	Tukey-K	0.619
T7	3	AgeCat*Trt*Glucose	Young Glu p	G0 G1	-0.2	1.1	165	-0.2	0.857	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Young Glu p	G0 G2	0.817	1.1	165	0.73	0.465	Tukey-K	0.978
T7	3	AgeCat*Trt*Glucose	Young Glu p	G0 G3	-0.52	1.1	165	-0.5	0.644	Tukey-K	0.997
T7	3	AgeCat*Trt*Glucose	Young Glu p	G0 G4	-0.34	1.1	165	-0.3	0.758	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Young Glu p	G0 G5	0.095	1.1	165	0.09	0.932	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Young Glu p	G1 G2	1.019	1.1	165	0.91	0.363	Tukey-K	0.943
T7	3	AgeCat*Trt*Glucose	Young Glu p	G1 G3	-0.32	1.1	165	-0.3	0.778	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Young Glu p	G1 G4	-0.14	1.1	165	-0.1	0.899	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Young Glu p	G1 G5	0.297	1.1	165	0.27	0.791	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Young Glu p	G2 G3	-1.33	1.1	165	-1.2	0.234	Tukey-K	0.839
T7	3	AgeCat*Trt*Glucose	Young Glu p	G2 G4	-1.16	1.1	165	-1	0.3	Tukey-K	0.904
T7	3	AgeCat*Trt*Glucose	Young Glu p	G2 G5	-0.72	1.1	165	-0.6	0.519	Tukey-K	0.987
T7	3	AgeCat*Trt*Glucose	Young Glu p	G3 G4	0.173	1.1	165	0.16	0.877	Tukey-K	1
T7	3	AgeCat*Trt*Glucose	Young Glu p	G3 G5	0.613	1.1	165	0.55	0.584	Tukey-K	0.994
T7	3	AgeCat*Trt*Glucose	Young Glu p	G4 G5	0.439	1.1	165	0.39	0.695	Tukey-K	0.999
T8	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G1	-0.52	1.5	165	-0.3	0.736	Tukey-K	0.999
T8	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G2	0.896	1.5	165	0.58	0.562	Tukey-K	0.992
T8	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G3	0.844	1.5	165	0.55	0.585	Tukey-K	0.994
T8	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G4	-1.38	1.5	165	-0.9	0.371	Tukey-K	0.947
T8	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G5	-0.74	1.5	165	-0.5	0.632	Tukey-K	0.997
T8	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G2	1.418	1.5	165	0.92	0.359	Tukey-K	0.941
T8	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G3	1.366	1.5	165	0.89	0.377	Tukey-K	0.95
T8	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G4	-0.86	1.5	165	-0.6	0.578	Tukey-K	0.993
T8	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G5	-0.22	1.5	165	-0.1	0.888	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G3	-0.05	1.5	165	-0	0.973	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G4	-2.28	1.5	165	-1.5	0.142	Tukey-K	0.679
T8	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G5	-1.64	1.5	165	-1.1	0.291	Tukey-K	0.896

T8	3	AgeCat*Trt*Glucose	Middle Glu m	G3 G4	-2.23	1.5	165	-1.4	0.151	Tukey-K	0.7
T8	3	AgeCat*Trt*Glucose	Middle Glu m	G3 G5	-1.58	1.5	165	-1	0.306	Tukey-K	0.908
T8	3	AgeCat*Trt*Glucose	Middle Glu m	G4 G5	0.643	1.5	165	0.42	0.677	Tukey-K	0.998
T8	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G1	1.342	1.5	165	0.87	0.386	Tukey-K	0.953
T8	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G2	1.026	1.5	165	0.67	0.507	Tukey-K	0.985
T8	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G3	2.628	1.5	165	1.7	0.09	Tukey-K	0.531
T8	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G4	0.908	1.5	165	0.59	0.557	Tukey-K	0.992
T8	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G5	0.113	1.5	165	0.07	0.942	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G2	-0.32	1.5	165	-0.2	0.838	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G3	1.286	1.5	165	0.83	0.405	Tukey-K	0.961
T8	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G4	-0.43	1.5	165	-0.3	0.779	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G5	-1.23	1.5	165	-0.8	0.427	Tukey-K	0.968
T8	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G3	1.603	1.5	165	1.04	0.3	Tukey-K	0.904
T8	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G4	-0.12	1.5	165	-0.1	0.939	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G5	-0.91	1.5	165	-0.6	0.555	Tukey-K	0.991
T8	3	AgeCat*Trt*Glucose	Middle Glu p	G3 G4	-1.72	1.5	165	-1.1	0.266	Tukey-K	0.874
T8	3	AgeCat*Trt*Glucose	Middle Glu p	G3 G5	-2.52	1.5	165	-1.6	0.105	Tukey-K	0.579
T8	3	AgeCat*Trt*Glucose	Middle Glu p	G4 G5	-0.8	1.5	165	-0.5	0.607	Tukey-K	0.996
T8	3	AgeCat*Trt*Glucose	Old Glu m	G0 G1	0.253	1.1	165	0.23	0.817	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Old Glu m	G0 G2	-0.74	1.1	165	-0.7	0.498	Tukey-K	0.984
T8	3	AgeCat*Trt*Glucose	Old Glu m	G0 G3	-1.17	1.1	165	-1.1	0.283	Tukey-K	0.89
T8	3	AgeCat*Trt*Glucose	Old Glu m	G0 G4	0.266	1.1	165	0.24	0.808	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Old Glu m	G0 G5	-0.31	1.1	165	-0.3	0.773	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Old Glu m	G1 G2	-0.99	1.1	165	-0.9	0.363	Tukey-K	0.943
T8	3	AgeCat*Trt*Glucose	Old Glu m	G1 G3	-1.43	1.1	165	-1.3	0.192	Tukey-K	0.78
T8	3	AgeCat*Trt*Glucose	Old Glu m	G1 G4	0.013	1.1	165	0.01	0.991	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Old Glu m	G1 G5	-0.57	1.1	165	-0.5	0.603	Tukey-K	0.995
T8	3	AgeCat*Trt*Glucose	Old Glu m	G2 G3	-0.43	1.1	165	-0.4	0.691	Tukey-K	0.999
T8	3	AgeCat*Trt*Glucose	Old Glu m	G2 G4	1.007	1.1	165	0.92	0.357	Tukey-K	0.94
T8	3	AgeCat*Trt*Glucose	Old Glu m	G2 G5	0.426	1.1	165	0.39	0.696	Tukey-K	0.999
T8	3	AgeCat*Trt*Glucose	Old Glu m	G3 G4	1.44	1.1	165	1.32	0.188	Tukey-K	0.773
T8	3	AgeCat*Trt*Glucose	Old Glu m	G3 G5	0.86	1.1	165	0.79	0.431	Tukey-K	0.969
T8	3	AgeCat*Trt*Glucose	Old Glu m	G4 G5	-0.58	1.1	165	-0.5	0.595	Tukey-K	0.995
T8	3	AgeCat*Trt*Glucose	Old Glu p	G0 G1	1.023	1.1	165	0.94	0.349	Tukey-K	0.936
T8	3	AgeCat*Trt*Glucose	Old Glu p	G0 G2	2.448	1.1	165	2.25	0.026	Tukey-K	0.223
T8	3	AgeCat*Trt*Glucose	Old Glu p	G0 G3	2.768	1.1	165	2.54	0.012	Tukey-K	0.119
T8	3	AgeCat*Trt*Glucose	Old Glu p	G0 G4	0.914	1.1	165	0.84	0.403	Tukey-K	0.96
T8	3	AgeCat*Trt*Glucose	Old Glu p	G0 G5	1.095	1.1	165	1	0.317	Tukey-K	0.916
T8	3	AgeCat*Trt*Glucose	Old Glu p	G1 G2	1.425	1.1	165	1.31	0.193	Tukey-K	0.781
T8	3	AgeCat*Trt*Glucose	Old Glu p	G1 G3	1.744	1.1	165	1.6	0.112	Tukey-K	0.6
T8	3	AgeCat*Trt*Glucose	Old Glu p	G1 G4	-0.11	1.1	165	-0.1	0.92	Tukey-K	1

T8	3	AgeCat*Trt*Glucose	Old Glu p	G1 G5	0.072	1.1	165	0.07	0.948	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Old Glu p	G2 G3	0.319	1.1	165	0.29	0.77	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Old Glu p	G2 G4	-1.53	1.1	165	-1.4	0.161	Tukey-K	0.723
T8	3	AgeCat*Trt*Glucose	Old Glu p	G2 G5	-1.35	1.1	165	-1.2	0.216	Tukey-K	0.816
T8	3	AgeCat*Trt*Glucose	Old Glu p	G3 G4	-1.85	1.1	165	-1.7	0.091	Tukey-K	0.534
T8	3	AgeCat*Trt*Glucose	Old Glu p	G3 G5	-1.67	1.1	165	-1.5	0.127	Tukey-K	0.643
T8	3	AgeCat*Trt*Glucose	Old Glu p	G4 G5	0.182	1.1	165	0.17	0.868	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Young Glu m	G0 G1	-0.09	1.3	165	-0.1	0.943	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Young Glu m	G0 G2	-0.95	1.3	165	-0.8	0.452	Tukey-K	0.975
T8	3	AgeCat*Trt*Glucose	Young Glu m	G0 G3	-1.19	1.3	165	-0.9	0.347	Tukey-K	0.935
T8	3	AgeCat*Trt*Glucose	Young Glu m	G0 G4	-1.83	1.3	165	-1.5	0.147	Tukey-K	0.693
T8	3	AgeCat*Trt*Glucose	Young Glu m	G0 G5	0.043	1.3	165	0.03	0.973	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Young Glu m	G1 G2	-0.86	1.3	165	-0.7	0.496	Tukey-K	0.984
T8	3	AgeCat*Trt*Glucose	Young Glu m	G1 G3	-1.1	1.3	165	-0.9	0.385	Tukey-K	0.953
T8	3	AgeCat*Trt*Glucose	Young Glu m	G1 G4	-1.74	1.3	165	-1.4	0.168	Tukey-K	0.737
T8	3	AgeCat*Trt*Glucose	Young Glu m	G1 G5	0.133	1.3	165	0.11	0.916	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Young Glu m	G2 G3	-0.24	1.3	165	-0.2	0.85	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Young Glu m	G2 G4	-0.88	1.3	165	-0.7	0.484	Tukey-K	0.982
T8	3	AgeCat*Trt*Glucose	Young Glu m	G2 G5	0.992	1.3	165	0.79	0.432	Tukey-K	0.969
T8	3	AgeCat*Trt*Glucose	Young Glu m	G3 G4	-0.65	1.3	165	-0.5	0.609	Tukey-K	0.996
T8	3	AgeCat*Trt*Glucose	Young Glu m	G3 G5	1.23	1.3	165	0.98	0.33	Tukey-K	0.925
T8	3	AgeCat*Trt*Glucose	Young Glu m	G4 G5	1.875	1.3	165	1.49	0.138	Tukey-K	0.672
T8	3	AgeCat*Trt*Glucose	Young Glu p	G0 G1	-1.19	1.3	165	-0.9	0.345	Tukey-K	0.933
T8	3	AgeCat*Trt*Glucose	Young Glu p	G0 G2	-2.14	1.3	165	-1.7	0.091	Tukey-K	0.532
T8	3	AgeCat*Trt*Glucose	Young Glu p	G0 G3	-1.67	1.3	165	-1.3	0.188	Tukey-K	0.772
T8	3	AgeCat*Trt*Glucose	Young Glu p	G0 G4	-1.52	1.3	165	-1.2	0.23	Tukey-K	0.834
T8	3	AgeCat*Trt*Glucose	Young Glu p	G0 G5	-1.46	1.3	165	-1.2	0.247	Tukey-K	0.854
T8	3	AgeCat*Trt*Glucose	Young Glu p	G1 G2	-0.95	1.3	165	-0.8	0.451	Tukey-K	0.974
T8	3	AgeCat*Trt*Glucose	Young Glu p	G1 G3	-0.47	1.3	165	-0.4	0.708	Tukey-K	0.999
T8	3	AgeCat*Trt*Glucose	Young Glu p	G1 G4	-0.32	1.3	165	-0.3	0.797	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Young Glu p	G1 G5	-0.27	1.3	165	-0.2	0.831	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Young Glu p	G2 G3	0.477	1.3	165	0.38	0.705	Tukey-K	0.999
T8	3	AgeCat*Trt*Glucose	Young Glu p	G2 G4	0.627	1.3	165	0.5	0.619	Tukey-K	0.996
T8	3	AgeCat*Trt*Glucose	Young Glu p	G2 G5	0.681	1.3	165	0.54	0.589	Tukey-K	0.994
T8	3	AgeCat*Trt*Glucose	Young Glu p	G3 G4	0.149	1.3	165	0.12	0.906	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Young Glu p	G3 G5	0.203	1.3	165	0.16	0.872	Tukey-K	1
T8	3	AgeCat*Trt*Glucose	Young Glu p	G4 G5	0.054	1.3	165	0.04	0.966	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G1	0.575	1.4	165	0.41	0.682	Tukey-K	0.998
T9	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G2	1.236	1.4	165	0.88	0.378	Tukey-K	0.95
T9	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G3	1.319	1.4	165	0.94	0.347	Tukey-K	0.935
T9	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G4	-1.05	1.4	165	-0.8	0.453	Tukey-K	0.975

T9	3	AgeCat*Trt*Glucose	Middle Glu m	G0 G5	1.919	1.4	165	1.37	0.172	Tukey-K	0.744
T9	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G2	0.661	1.4	165	0.47	0.637	Tukey-K	0.997
T9	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G3	0.744	1.4	165	0.53	0.595	Tukey-K	0.995
T9	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G4	-1.63	1.4	165	-1.2	0.246	Tukey-K	0.854
T9	3	AgeCat*Trt*Glucose	Middle Glu m	G1 G5	1.344	1.4	165	0.96	0.338	Tukey-K	0.93
T9	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G3	0.083	1.4	165	0.06	0.953	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G4	-2.29	1.4	165	-1.6	0.104	Tukey-K	0.576
T9	3	AgeCat*Trt*Glucose	Middle Glu m	G2 G5	0.683	1.4	165	0.49	0.626	Tukey-K	0.997
T9	3	AgeCat*Trt*Glucose	Middle Glu m	G3 G4	-2.37	1.4	165	-1.7	0.092	Tukey-K	0.537
T9	3	AgeCat*Trt*Glucose	Middle Glu m	G3 G5	0.599	1.4	165	0.43	0.669	Tukey-K	0.998
T9	3	AgeCat*Trt*Glucose	Middle Glu m	G4 G5	2.971	1.4	165	2.12	0.035	Tukey-K	0.28
T9	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G1	1.181	1.4	165	0.84	0.4	Tukey-K	0.959
T9	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G2	1.153	1.4	165	0.82	0.411	Tukey-K	0.963
T9	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G3	1.493	1.4	165	1.07	0.287	Tukey-K	0.894
T9	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G4	1.443	1.4	165	1.03	0.304	Tukey-K	0.907
T9	3	AgeCat*Trt*Glucose	Middle Glu p	G0 G5	0.209	1.4	165	0.15	0.882	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G2	-0.03	1.4	165	-0	0.984	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G3	0.311	1.4	165	0.22	0.824	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G4	0.261	1.4	165	0.19	0.852	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Middle Glu p	G1 G5	-0.97	1.4	165	-0.7	0.488	Tukey-K	0.982
T9	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G3	0.34	1.4	165	0.24	0.808	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G4	0.29	1.4	165	0.21	0.836	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Middle Glu p	G2 G5	-0.94	1.4	165	-0.7	0.501	Tukey-K	0.984
T9	3	AgeCat*Trt*Glucose	Middle Glu p	G3 G4	-0.05	1.4	165	-0	0.972	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Middle Glu p	G3 G5	-1.28	1.4	165	-0.9	0.36	Tukey-K	0.941
T9	3	AgeCat*Trt*Glucose	Middle Glu p	G4 G5	-1.23	1.4	165	-0.9	0.379	Tukey-K	0.95
T9	3	AgeCat*Trt*Glucose	Old Glu m	G0 G1	0.208	1	165	0.21	0.834	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Old Glu m	G0 G2	-0.12	1	165	-0.1	0.902	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Old Glu m	G0 G3	-0.29	1	165	-0.3	0.771	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Old Glu m	G0 G4	-0.66	1	165	-0.7	0.506	Tukey-K	0.985
T9	3	AgeCat*Trt*Glucose	Old Glu m	G0 G5	-0.23	1	165	-0.2	0.819	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Old Glu m	G1 G2	-0.33	1	165	-0.3	0.74	Tukey-K	0.999
T9	3	AgeCat*Trt*Glucose	Old Glu m	G1 G3	-0.5	1	165	-0.5	0.617	Tukey-K	0.996
T9	3	AgeCat*Trt*Glucose	Old Glu m	G1 G4	-0.87	1	165	-0.9	0.382	Tukey-K	0.952
T9	3	AgeCat*Trt*Glucose	Old Glu m	G1 G5	-0.43	1	165	-0.4	0.661	Tukey-K	0.998
T9	3	AgeCat*Trt*Glucose	Old Glu m	G2 G3	-0.17	1	165	-0.2	0.867	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Old Glu m	G2 G4	-0.54	1	165	-0.5	0.587	Tukey-K	0.994
T9	3	AgeCat*Trt*Glucose	Old Glu m	G2 G5	-0.11	1	165	-0.1	0.915	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Old Glu m	G3 G4	-0.37	1	165	-0.4	0.708	Tukey-K	0.999
T9	3	AgeCat*Trt*Glucose	Old Glu m	G3 G5	0.061	1	165	0.06	0.951	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Old Glu m	G4 G5	0.432	1	165	0.44	0.663	Tukey-K	0.998

T9	3	AgeCat*Trt*Glucose	Old Glu p	G0 G1	0.945	1	165	0.96	0.341	Tukey-K	0.931
T9	3	AgeCat*Trt*Glucose	Old Glu p	G0 G2	2.658	1	165	2.69	0.008	Tukey-K	0.083
T9	3	AgeCat*Trt*Glucose	Old Glu p	G0 G3	1.362	1	165	1.38	0.17	Tukey-K	0.741
T9	3	AgeCat*Trt*Glucose	Old Glu p	G0 G4	0.476	1	165	0.48	0.631	Tukey-K	0.997
T9	3	AgeCat*Trt*Glucose	Old Glu p	G0 G5	0.208	1	165	0.21	0.834	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Old Glu p	G1 G2	1.713	1	165	1.73	0.085	Tukey-K	0.513
T9	3	AgeCat*Trt*Glucose	Old Glu p	G1 G3	0.417	1	165	0.42	0.674	Tukey-K	0.998
T9	3	AgeCat*Trt*Glucose	Old Glu p	G1 G4	-0.47	1	165	-0.5	0.636	Tukey-K	0.997
T9	3	AgeCat*Trt*Glucose	Old Glu p	G1 G5	-0.74	1	165	-0.7	0.457	Tukey-K	0.976
T9	3	AgeCat*Trt*Glucose	Old Glu p	G2 G3	-1.3	1	165	-1.3	0.192	Tukey-K	0.779
T9	3	AgeCat*Trt*Glucose	Old Glu p	G2 G4	-2.18	1	165	-2.2	0.029	Tukey-K	0.241
T9	3	AgeCat*Trt*Glucose	Old Glu p	G2 G5	-2.45	1	165	-2.5	0.014	Tukey-K	0.137
T9	3	AgeCat*Trt*Glucose	Old Glu p	G3 G4	-0.89	1	165	-0.9	0.372	Tukey-K	0.947
T9	3	AgeCat*Trt*Glucose	Old Glu p	G3 G5	-1.15	1	165	-1.2	0.245	Tukey-K	0.852
T9	3	AgeCat*Trt*Glucose	Old Glu p	G4 G5	-0.27	1	165	-0.3	0.786	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Young Glu m	G0 G1	-0.48	1.1	165	-0.4	0.675	Tukey-K	0.998
T9	3	AgeCat*Trt*Glucose	Young Glu m	G0 G2	-1.03	1.1	165	-0.9	0.37	Tukey-K	0.946
T9	3	AgeCat*Trt*Glucose	Young Glu m	G0 G3	-1.39	1.1	165	-1.2	0.224	Tukey-K	0.826
T9	3	AgeCat*Trt*Glucose	Young Glu m	G0 G4	-0.1	1.1	165	-0.1	0.929	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Young Glu m	G0 G5	-0.27	1.1	165	-0.2	0.816	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Young Glu m	G1 G2	-0.55	1.1	165	-0.5	0.632	Tukey-K	0.997
T9	3	AgeCat*Trt*Glucose	Young Glu m	G1 G3	-0.91	1.1	165	-0.8	0.425	Tukey-K	0.967
T9	3	AgeCat*Trt*Glucose	Young Glu m	G1 G4	0.378	1.1	165	0.33	0.741	Tukey-K	0.999
T9	3	AgeCat*Trt*Glucose	Young Glu m	G1 G5	0.214	1.1	165	0.19	0.852	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Young Glu m	G2 G3	-0.37	1.1	165	-0.3	0.749	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Young Glu m	G2 G4	0.926	1.1	165	0.81	0.419	Tukey-K	0.965
T9	3	AgeCat*Trt*Glucose	Young Glu m	G2 G5	0.762	1.1	165	0.67	0.506	Tukey-K	0.985
T9	3	AgeCat*Trt*Glucose	Young Glu m	G3 G4	1.292	1.1	165	1.13	0.26	Tukey-K	0.868
T9	3	AgeCat*Trt*Glucose	Young Glu m	G3 G5	1.128	1.1	165	0.99	0.325	Tukey-K	0.921
T9	3	AgeCat*Trt*Glucose	Young Glu m	G4 G5	-0.16	1.1	165	-0.1	0.886	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Young Glu p	G0 G1	-2.36	1.1	165	-2.1	0.04	Tukey-K	0.31
T9	3	AgeCat*Trt*Glucose	Young Glu p	G0 G2	-2.29	1.1	165	-2	0.046	Tukey-K	0.343
T9	3	AgeCat*Trt*Glucose	Young Glu p	G0 G3	-1.97	1.1	165	-1.7	0.086	Tukey-K	0.515
T9	3	AgeCat*Trt*Glucose	Young Glu p	G0 G4	-2.95	1.1	165	-2.6	0.011	Tukey-K	0.108
T9	3	AgeCat*Trt*Glucose	Young Glu p	G0 G5	-1.33	1.1	165	-1.2	0.245	Tukey-K	0.851
T9	3	AgeCat*Trt*Glucose	Young Glu p	G1 G2	0.069	1.1	165	0.06	0.952	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Young Glu p	G1 G3	0.387	1.1	165	0.34	0.735	Tukey-K	0.999
T9	3	AgeCat*Trt*Glucose	Young Glu p	G1 G4	-0.59	1.1	165	-0.5	0.609	Tukey-K	0.996
T9	3	AgeCat*Trt*Glucose	Young Glu p	G1 G5	1.027	1.1	165	0.9	0.37	Tukey-K	0.946
T9	3	AgeCat*Trt*Glucose	Young Glu p	G2 G3	0.318	1.1	165	0.28	0.781	Tukey-K	1
T9	3	AgeCat*Trt*Glucose	Young Glu p	G2 G4	-0.65	1.1	165	-0.6	0.568	Tukey-K	0.993

T9	3	AgeCat*Trt*Glucose	Young Glu p	G2 G5	0.958	1.1	165	0.84	0.403	Tukey-K	0.96
T9	3	AgeCat*Trt*Glucose	Young Glu p	G3 G4	-0.97	1.1	165	-0.9	0.396	Tukey-K	0.957
T9	3	AgeCat*Trt*Glucose	Young Glu p	G3 G5	0.64	1.1	165	0.56	0.576	Tukey-K	0.993
T9	3	AgeCat*Trt*Glucose	Young Glu p	G4 G5	1.613	1.1	165	1.41	0.16	Tukey-K	0.72

**TEST 5:** Gave the differences between -FBS/- 30nM glucose and +FBS/+ 30 nM glucose in specific age and specific treatment:

Gene	Slice	Glu	_Glu	Estimate	StdErr	DF	tValue	Raw_p	fdr_p
T1	AgeCat Middle Trt G0	m	p	0.2106	0.8993	165	0.2342	0.8151	0.8631
T1	AgeCat Middle Trt G1	m	p	1.3148	0.8993	165	1.4621	0.1456	0.7223
T1	AgeCat Middle Trt G2	m	p	-0.47	0.8993	165	-0.523	0.6019	0.7223
T1	AgeCat Middle Trt G3	m	p	-1.067	0.8993	165	-1.186	0.2372	0.7223
T1	AgeCat Middle Trt G4	m	p	1.1127	0.8993	165	1.2374	0.2177	0.7223
T1	AgeCat Middle Trt G5	m	p	-1.143	0.8993	165	-1.27	0.2057	0.7223
T1	AgeCat Old Trt G0	m	p	-0.7	0.6359	165	-1.1	0.2728	0.7223
T1	AgeCat Old Trt G1	m	p	-0.479	0.6359	165	-0.753	0.4523	0.7223
T1	AgeCat Old Trt G2	m	p	-0.488	0.6359	165	-0.767	0.4441	0.7223
T1	AgeCat Old Trt G3	m	p	-0.048	0.6359	165	-0.075	0.9402	0.9402
T1	AgeCat Old Trt G4	m	p	-0.385	0.6359	165	-0.606	0.5454	0.7223
T1	AgeCat Old Trt G5	m	p	-0.847	0.6359	165	-1.332	0.1846	0.7223
T1	AgeCat Young Trt G0	m	p	-0.579	0.7342	165	-0.789	0.4314	0.7223
T1	AgeCat Young Trt G1	m	p	0.5692	0.7342	165	0.7752	0.4393	0.7223
T1	AgeCat Young Trt G2	m	p	-0.437	0.7342	165	-0.595	0.5525	0.7223
T1	AgeCat Young Trt G3	m	p	0.5963	0.7342	165	0.8121	0.4179	0.7223
T1	AgeCat Young Trt G4	m	p	-0.411	0.7342	165	-0.56	0.5766	0.7223
T1	AgeCat Young Trt G5	m	p	-0.201	0.7342	165	-0.274	0.7848	0.8631
T10	AgeCat Middle Trt G0	m	p	-1.818	1.2099	165	-1.503	0.1348	0.4673
T10	AgeCat Middle Trt G1	m	p	-0.081	1.2099	165	-0.067	0.9464	0.9464
T10	AgeCat Middle Trt G2	m	p	-0.225	1.2099	165	-0.186	0.8527	0.9382
T10	AgeCat Middle Trt G3	m	p	-1.429	1.2099	165	-1.181	0.2392	0.4673
T10	AgeCat Middle Trt G4	m	p	1.3869	1.2099	165	1.1462	0.2534	0.4673
T10	AgeCat Middle Trt G5	m	p	-1.488	1.2099	165	-1.229	0.2207	0.4673
T10	AgeCat Old Trt G0	m	p	-1.967	0.8556	165	-2.299	0.0228	0.4101
T10	AgeCat Old Trt G1	m	p	-1.497	0.8556	165	-1.75	0.082	0.4673
T10	AgeCat Old Trt G2	m	p	1.0866	0.8556	165	1.27	0.2059	0.4673
T10	AgeCat Old Trt G3	m	p	0.1228	0.8556	165	0.1435	0.886	0.9382
T10	AgeCat Old Trt G4	m	p	-1.108	0.8556	165	-1.296	0.1969	0.4673
T10	AgeCat Old Trt G5	m	p	-0.206	0.8556	165	-0.241	0.8101	0.9382
T10	AgeCat Young Trt G0	m	p	-0.555	0.9879	165	-0.562	0.5751	0.7394
T10	AgeCat Young Trt G1	m	p	-0.911	0.9879	165	-0.922	0.3579	0.5856
T10	AgeCat Young Trt G2	m	p	-0.663	0.9879	165	-0.671	0.5029	0.6963
T10	AgeCat Young Trt G3	m	p	-0.68	0.9879	165	-0.688	0.4925	0.6963
T10	AgeCat Young Trt G4	m	p	-1.118	0.9879	165	-1.131	0.2596	0.4673
T10	AgeCat Young Trt G5	m	p	-1.68	0.9879	165	-1.701	0.0909	0.4673
T2	AgeCat Middle Trt G0	m	p	0.4956	0.9585	165	0.5171	0.6058	0.7269
T2	AgeCat Middle Trt G1	m	p	0.8685	0.9585	165	0.9062	0.3662	0.6506
T2	AgeCat Middle Trt G2	m	p	-1.761	0.9585	165	-1.838	0.0679	0.4033

T2	AgeCat Middle Trt G3	m	p	-0.642	0.9585	165	-0.67	0.504	0.6978
T2	AgeCat Middle Trt G4	m	p	1.0952	0.9585	165	1.1427	0.2548	0.6506
T2	AgeCat Middle Trt G5	m	p	-0.871	0.9585	165	-0.909	0.3647	0.6506
T2	AgeCat Old Trt G0	m	p	-1.423	0.6777	165	-2.099	0.0373	0.4033
T2	AgeCat Old Trt G1	m	p	-1.36	0.6777	165	-2.007	0.0464	0.4033
T2	AgeCat Old Trt G2	m	p	-0.723	0.6777	165	-1.067	0.2873	0.6506
T2	AgeCat Old Trt G3	m	p	-0.198	0.6777	165	-0.292	0.7708	0.8161
T2	AgeCat Old Trt G4	m	p	-0.382	0.6777	165	-0.563	0.5742	0.7269
T2	AgeCat Old Trt G5	m	p	-1.157	0.6777	165	-1.707	0.0896	0.4033
T2	AgeCat Young Trt G0	m	p	-0.641	0.7826	165	-0.819	0.414	0.6506
T2	AgeCat Young Trt G1	m	p	0.6142	0.7826	165	0.7848	0.4337	0.6506
T2	AgeCat Young Trt G2	m	p	-0.933	0.7826	165	-1.192	0.2349	0.6506
T2	AgeCat Young Trt G3	m	p	-0.013	0.7826	165	-0.017	0.9869	0.9869
T2	AgeCat Young Trt G4	m	p	-0.345	0.7826	165	-0.441	0.6599	0.7424
T2	AgeCat Young Trt G5	m	p	-0.824	0.7826	165	-1.053	0.2938	0.6506
T3	AgeCat Middle Trt G0	m	p	0.6644	0.923	165	0.7198	0.4727	0.709
T3	AgeCat Middle Trt G1	m	p	1.3185	0.923	165	1.4285	0.155	0.4651
T3	AgeCat Middle Trt G2	m	p	0.1313	0.923	165	0.1422	0.8871	0.9266
T3	AgeCat Middle Trt G3	m	p	-1.801	0.923	165	-1.951	0.0528	0.4378
T3	AgeCat Middle Trt G4	m	p	1.3465	0.923	165	1.4587	0.1465	0.4651
T3	AgeCat Middle Trt G5	m	p	-0.981	0.923	165	-1.063	0.2893	0.4734
T3	AgeCat Old Trt G0	m	p	-1.178	0.6527	165	-1.805	0.073	0.4378
T3	AgeCat Old Trt G1	m	p	-1.412	0.6527	165	-2.163	0.032	0.4378
T3	AgeCat Old Trt G2	m	p	-0.727	0.6527	165	-1.113	0.2673	0.4734
T3	AgeCat Old Trt G3	m	p	0.1641	0.6527	165	0.2514	0.8018	0.9266
T3	AgeCat Old Trt G4	m	p	-0.764	0.6527	165	-1.171	0.2434	0.4734
T3	AgeCat Old Trt G5	m	p	-1.032	0.6527	165	-1.58	0.1159	0.4651
T3	AgeCat Young Trt G0	m	p	0.2408	0.7537	165	0.3195	0.7497	0.9266
T3	AgeCat Young Trt G1	m	p	0.8525	0.7537	165	1.1311	0.2596	0.4734
T3	AgeCat Young Trt G2	m	p	0.0696	0.7537	165	0.0923	0.9266	0.9266
T3	AgeCat Young Trt G3	m	p	0.1021	0.7537	165	0.1354	0.8924	0.9266
T3	AgeCat Young Trt G4	m	p	0.08	0.7537	165	0.1061	0.9156	0.9266
T3	AgeCat Young Trt G5	m	p	-0.828	0.7537	165	-1.099	0.2733	0.4734
T4	AgeCat Middle Trt G0	m	p	0.0256	0.9742	165	0.0263	0.979	0.979
T4	AgeCat Middle Trt G1	m	p	1.1948	0.9742	165	1.2264	0.2218	0.8584
T4	AgeCat Middle Trt G2	m	p	-0.747	0.9742	165	-0.767	0.444	0.979
T4	AgeCat Middle Trt G3	m	p	-1.214	0.9742	165	-1.246	0.2144	0.8584
T4	AgeCat Middle Trt G4	m	p	1.1527	0.9742	165	1.1832	0.2384	0.8584
T4	AgeCat Middle Trt G5	m	p	-1.299	0.9742	165	-1.333	0.1843	0.8584
T4	AgeCat Old Trt G0	m	p	-0.235	0.6889	165	-0.341	0.7338	0.979
T4	AgeCat Old Trt G1	m	p	-1.318	0.6889	165	-1.914	0.0574	0.8584
T4	AgeCat Old Trt G2	m	p	0.0353	0.6889	165	0.0513	0.9592	0.979
T4	AgeCat Old Trt G3	m	p	0.0722	0.6889	165	0.1048	0.9167	0.979

T4	AgeCat Old Trt G4	m	p	-0.56	0.6889	165	-0.812	0.4177	0.979
T4	AgeCat Old Trt G5	m	p	-0.31	0.6889	165	-0.45	0.6536	0.979
T4	AgeCat Young Trt G0	m	p	-0.648	0.7955	165	-0.814	0.4168	0.979
T4	AgeCat Young Trt G1	m	p	-0.245	0.7955	165	-0.308	0.7585	0.979
T4	AgeCat Young Trt G2	m	p	0.0721	0.7955	165	0.0906	0.9279	0.979
T4	AgeCat Young Trt G3	m	p	0.1929	0.7955	165	0.2425	0.8087	0.979
T4	AgeCat Young Trt G4	m	p	-0.354	0.7955	165	-0.445	0.6567	0.979
T4	AgeCat Young Trt G5	m	p	-0.187	0.7955	165	-0.236	0.8139	0.979
T5	AgeCat Middle Trt G0	m	p	1.0981	1.0392	165	1.0567	0.2922	0.9601
T5	AgeCat Middle Trt G1	m	p	0.741	1.0392	165	0.7131	0.4768	0.9601
T5	AgeCat Middle Trt G2	m	p	0.4562	1.0392	165	0.439	0.6612	0.9601
T5	AgeCat Middle Trt G3	m	p	-0.582	1.0392	165	-0.56	0.5763	0.9601
T5	AgeCat Middle Trt G4	m	p	1.0302	1.0392	165	0.9913	0.323	0.9601
T5	AgeCat Middle Trt G5	m	p	-0.74	1.0392	165	-0.712	0.4774	0.9601
T5	AgeCat Old Trt G0	m	p	0.0666	0.7349	165	0.0906	0.9279	0.9601
T5	AgeCat Old Trt G1	m	p	-1.199	0.7349	165	-1.632	0.1046	0.9601
T5	AgeCat Old Trt G2	m	p	0.2203	0.7349	165	0.2998	0.7647	0.9601
T5	AgeCat Old Trt G3	m	p	0.1422	0.7349	165	0.1935	0.8468	0.9601
T5	AgeCat Old Trt G4	m	p	-0.474	0.7349	165	-0.645	0.5198	0.9601
T5	AgeCat Old Trt G5	m	p	-0.51	0.7349	165	-0.694	0.4889	0.9601
T5	AgeCat Young Trt G0	m	p	0.0425	0.8485	165	0.0501	0.9601	0.9601
T5	AgeCat Young Trt G1	m	p	-0.136	0.8485	165	-0.16	0.873	0.9601
T5	AgeCat Young Trt G2	m	p	-0.132	0.8485	165	-0.156	0.8765	0.9601
T5	AgeCat Young Trt G3	m	p	0.2029	0.8485	165	0.2391	0.8113	0.9601
T5	AgeCat Young Trt G4	m	p	-1.057	0.8485	165	-1.245	0.2148	0.9601
T5	AgeCat Young Trt G5	m	p	-0.906	0.8485	165	-1.068	0.2873	0.9601
T6	AgeCat Middle Trt G0	m	p	-0.987	1.6088	165	-0.613	0.5404	0.9079
T6	AgeCat Middle Trt G1	m	p	1.4913	1.6088	165	0.9269	0.3553	0.9079
T6	AgeCat Middle Trt G2	m	p	-0.415	1.6088	165	-0.258	0.7968	0.9079
T6	AgeCat Middle Trt G3	m	p	-2.324	1.6088	165	-1.445	0.1504	0.9079
T6	AgeCat Middle Trt G4	m	p	1.0015	1.6088	165	0.6225	0.5345	0.9079
T6	AgeCat Middle Trt G5	m	p	-2.937	1.6088	165	-1.826	0.0697	0.9079
T6	AgeCat Old Trt G0	m	p	-1.109	1.1376	165	-0.975	0.331	0.9079
T6	AgeCat Old Trt G1	m	p	-1.271	1.1376	165	-1.117	0.2655	0.9079
T6	AgeCat Old Trt G2	m	p	-0.675	1.1376	165	-0.593	0.5539	0.9079
T6	AgeCat Old Trt G3	m	p	0.4722	1.1376	165	0.4151	0.6786	0.9079
T6	AgeCat Old Trt G4	m	p	0.0247	1.1376	165	0.0217	0.9827	0.9827
T6	AgeCat Old Trt G5	m	p	-0.205	1.1376	165	-0.18	0.8574	0.9079
T6	AgeCat Young Trt G0	m	p	0.9917	1.3136	165	0.7549	0.4514	0.9079
T6	AgeCat Young Trt G1	m	p	-0.365	1.3136	165	-0.278	0.7815	0.9079
T6	AgeCat Young Trt G2	m	p	0.2488	1.3136	165	0.1894	0.85	0.9079
T6	AgeCat Young Trt G3	m	p	0.5729	1.3136	165	0.4361	0.6633	0.9079
T6	AgeCat Young Trt G4	m	p	-0.606	1.3136	165	-0.461	0.6453	0.9079

T6	AgeCat Young Trt G5	m	p	0.4567	1.3136	165	0.3476	0.7285	0.9079
T7	AgeCat Middle Trt G0	m	p	0.1342	1.3676	165	0.0981	0.922	0.922
T7	AgeCat Middle Trt G1	m	p	1.056	1.3676	165	0.7722	0.4411	0.7814
T7	AgeCat Middle Trt G2	m	p	-1.342	1.3676	165	-0.982	0.3277	0.7814
T7	AgeCat Middle Trt G3	m	p	0.3781	1.3676	165	0.2765	0.7825	0.9002
T7	AgeCat Middle Trt G4	m	p	1.5256	1.3676	165	1.1155	0.2662	0.7814
T7	AgeCat Middle Trt G5	m	p	-0.441	1.3676	165	-0.323	0.7474	0.9002
T7	AgeCat Old Trt G0	m	p	-0.965	0.967	165	-0.998	0.32	0.7814
T7	AgeCat Old Trt G1	m	p	-1.606	0.967	165	-1.661	0.0987	0.7814
T7	AgeCat Old Trt G2	m	p	1.2447	0.967	165	1.2871	0.1999	0.7814
T7	AgeCat Old Trt G3	m	p	1.3128	0.967	165	1.3575	0.1765	0.7814
T7	AgeCat Old Trt G4	m	p	-0.298	0.967	165	-0.308	0.7585	0.9002
T7	AgeCat Old Trt G5	m	p	-0.732	0.967	165	-0.756	0.4504	0.7814
T7	AgeCat Young Trt G0	m	p	0.795	1.1167	165	0.712	0.4775	0.7814
T7	AgeCat Young Trt G1	m	p	-1.465	1.1167	165	-1.312	0.1914	0.7814
T7	AgeCat Young Trt G2	m	p	0.2112	1.1167	165	0.1892	0.8502	0.9002
T7	AgeCat Young Trt G3	m	p	-0.241	1.1167	165	-0.216	0.8292	0.9002
T7	AgeCat Young Trt G4	m	p	0.3442	1.1167	165	0.3082	0.7583	0.9002
T7	AgeCat Young Trt G5	m	p	-0.97	1.1167	165	-0.869	0.3863	0.7814
T8	AgeCat Middle Trt G0	m	p	-0.863	1.5421	165	-0.56	0.5764	0.8022
T8	AgeCat Middle Trt G1	m	p	0.9998	1.5421	165	0.6483	0.5177	0.8022
T8	AgeCat Middle Trt G2	m	p	-0.734	1.5421	165	-0.476	0.6348	0.8022
T8	AgeCat Middle Trt G3	m	p	0.9206	1.5421	165	0.597	0.5513	0.8022
T8	AgeCat Middle Trt G4	m	p	1.4269	1.5421	165	0.9253	0.3562	0.7124
T8	AgeCat Middle Trt G5	m	p	-0.011	1.5421	165	-0.007	0.9942	0.9942
T8	AgeCat Old Trt G0	m	p	-1.878	1.0905	165	-1.722	0.0869	0.7124
T8	AgeCat Old Trt G1	m	p	-1.107	1.0905	165	-1.015	0.3114	0.7124
T8	AgeCat Old Trt G2	m	p	1.3116	1.0905	165	1.2028	0.2308	0.7124
T8	AgeCat Old Trt G3	m	p	2.0647	1.0905	165	1.8934	0.0601	0.7124
T8	AgeCat Old Trt G4	m	p	-1.23	1.0905	165	-1.128	0.2611	0.7124
T8	AgeCat Old Trt G5	m	p	-0.468	1.0905	165	-0.429	0.6685	0.8022
T8	AgeCat Young Trt G0	m	p	-0.117	1.2592	165	-0.093	0.9263	0.9808
T8	AgeCat Young Trt G1	m	p	-1.219	1.2592	165	-0.968	0.3343	0.7124
T8	AgeCat Young Trt G2	m	p	-1.311	1.2592	165	-1.041	0.2994	0.7124
T8	AgeCat Young Trt G3	m	p	-0.595	1.2592	165	-0.473	0.6369	0.8022
T8	AgeCat Young Trt G4	m	p	0.1992	1.2592	165	0.1582	0.8745	0.9808
T8	AgeCat Young Trt G5	m	p	-1.622	1.2592	165	-1.288	0.1996	0.7124
T9	AgeCat Middle Trt G0	m	p	-0.994	1.3987	165	-0.711	0.4783	0.7827
T9	AgeCat Middle Trt G1	m	p	-0.388	1.3987	165	-0.277	0.782	0.8797
T9	AgeCat Middle Trt G2	m	p	-1.078	1.3987	165	-0.77	0.4422	0.7827
T9	AgeCat Middle Trt G3	m	p	-0.821	1.3987	165	-0.587	0.5582	0.8373
T9	AgeCat Middle Trt G4	m	p	1.5006	1.3987	165	1.0729	0.2849	0.7827
T9	AgeCat Middle Trt G5	m	p	-2.704	1.3987	165	-1.933	0.0549	0.3296

T9	AgeCat Old Trt G0	m	p	-0.774	0.989	165	-0.783	0.4349	0.7827
T9	AgeCat Old Trt G1	m	p	-0.037	0.989	165	-0.037	0.9706	0.9706
T9	AgeCat Old Trt G2	m	p	2.0053	0.989	165	2.0276	0.0442	0.3296
T9	AgeCat Old Trt G3	m	p	0.8759	0.989	165	0.8857	0.3771	0.7827
T9	AgeCat Old Trt G4	m	p	0.3616	0.989	165	0.3656	0.7151	0.8786
T9	AgeCat Old Trt G5	m	p	-0.339	0.989	165	-0.343	0.7322	0.8786
T9	AgeCat Young Trt G0	m	p	0.0464	1.142	165	0.0406	0.9676	0.9706
T9	AgeCat Young Trt G1	m	p	-1.835	1.142	165	-1.607	0.11	0.495
T9	AgeCat Young Trt G2	m	p	-1.218	1.142	165	-1.067	0.2876	0.7827
T9	AgeCat Young Trt G3	m	p	-0.534	1.142	165	-0.467	0.6408	0.8786
T9	AgeCat Young Trt G4	m	p	-2.798	1.142	165	-2.45	0.0153	0.2757
T9	AgeCat Young Trt G5	m	p	-1.022	1.142	165	-0.895	0.3723	0.7827

## APPENDIX II: FOLD CHANGE

### KEY: GENES (T)

T1; AMPK $\alpha$ 1,

T2; AMPK $\alpha$ 2,

T3; AMPK $\beta$ 1,

T4; AMPK $\beta$ 2,

T5; AMPK $\gamma$ 1,

T6; AMPK $\gamma$ 1,

T7; AMPK $\gamma$ 3,

T8; IGF-1,

T9; GLUT4

T10; IR

### KEY: Treatments (G)

G0: Control

G1: Insulin

G2: human chorionic gonadotropin hCG

G3: EGF

G4: IGF-1

G5: Progesterone

### KEY: Media

m: minus (without FBS and glucose)

p: plus (without FBS and glucose).

**TEST 1:** Gave the ANOVA F-tests for each variable

Var	Effect	NumDF	DenDF	FValue	p-value
T10m	AgeCat	2	15	0.211429	0.81180
T10m	Trt	4	60	2.303104	0.06877
T10m	AgeCat* Treatment	8	60	0.802178	0.60306
T10p	AgeCat	2	14.86441	1.693066	0.21756
T10p	Treatment	4	58.93003	0.649981	0.62915
T10p	AgeCat* Treatment	8	58.9353	0.509224	0.84466
T1m	AgeCat	2	15	0.932055	0.41539
T1m	Treatment	4	60	0.830884	0.51084
T1m	AgeCat* Treatment	8	60	0.856651	0.55764
T1p	AgeCat	2	14.69823	0.82667	0.45685
T1p	Treatment	4	58.73118	0.975984	0.42765
T1p	AgeCat* Treatment	8	58.73173	0.55009	0.81380
T2m	AgeCat	2	15	3.026008	0.07870
T2m	Treatment	4	60	1.704006	0.16095
T2m	AgeCat* Treatment	8	60	0.921565	0.50535
T2p	AgeCat	2	15	1.261717	0.31156
T2p	Treatment	4	60	0.217748	0.92755
T2p	AgeCat* Treatment	8	60	1.216081	0.30536
T3m	AgeCat	2	15	1.057016	0.37200
T3m	Treatment	4	60	0.690918	0.60112
T3m	AgeCat* Treatment	8	60	0.549264	0.81455
T3p	AgeCat	2	15	0.287714	0.75402
T3p	Treatment	4	60	1.386159	0.24955
T3p	AgeCat* Treatment	8	60	1.665696	0.12570
T4m	AgeCat	2	15	0.210882	0.81223
T4m	Treatment	4	60	0.349397	0.84344
T4m	AgeCat* Treatment	8	60	0.811633	0.59510
T4p	AgeCat	2	15	0.929923	0.41618
T4p	Treatment	4	60	0.02788	0.99846
T4p	AgeCat* Treatment	8	60	0.475467	0.86874
T5m	AgeCat	2	12	0.424224	0.66372
T5m	Treatment	4	48	0.38278	0.81985
T5m	AgeCat* Treatment	8	48	0.479915	0.86435
T5p	AgeCat	2	12	1.184092	0.33937
T5p	Treatment	4	48	0.725806	0.57868
T5p	AgeCat* Treatment	8	48	0.910947	0.51556
T6m	AgeCat	2	14.4291	0.338082	0.71864
T6m	Treatment	4	53.04814	0.526133	0.71695

Var	Effect	NumDF	DenDF	FValue	p-value
T6m	AgeCat* Treatment	8	52.89547	0.659503	0.72422
T6p	AgeCat	2	13.02258	0.376947	0.69320
T6p	Treatment	4	51.05843	0.72371	0.57979
T6p	AgeCat* Treatment	8	51.06189	2.162163	0.04630
T7m	AgeCat	2	15.07685	0.71634	0.50443
T7m	Treatment	4	59.12579	2.559807	0.04774
T7m	AgeCat* Treatment	8	59.1049	0.700554	0.68966
T7p	AgeCat	2	15	0.1842	0.83362
T7p	Treatment	4	60	0.300503	0.87649
T7p	AgeCat* Treatment	8	60	0.885634	0.53401
T8m	AgeCat	2	15	0.050949	0.95049
T8m	Treatment	4	60	0.358771	0.83694
T8m	AgeCat* Treatment	8	60	0.900744	0.52187
T8p	AgeCat	2	15	3.340991	0.06309
T8p	Treatment	4	60	1.792275	0.14220
T8p	AgeCat* Treatment	8	60	0.722909	0.67057
T9m	AgeCat	2	15	0.377183	0.69211
T9m	Treatment	4	60	1.112481	0.35908
T9m	AgeCat* Treatment	8	60	1.289302	0.26634
T9p	AgeCat	2	15	2.671699	0.10174
T9p	Treatment	4	60	0.793092	0.53431
T9p	AgeCat* Treatment	8	60	0.623415	0.75483

- The log transform the data to help satisfy model assumptions
- The trans
- TEST 1: gives the ANOVA F-tests for each variable.
- TEST2: gives pairwise comparisons on main effects (Age Category or Age) for each var. or gene m, p combination.
- TESTS3: gives slice comparisons or interaction comparisons.
- Back trans formed means can be substantially lower than the means in the original scale, particularly when transformation is log

Transformation: adding a constant is required to take logs when there are zero's in the data

**TEST 2:** provided pairwise comparisons on main effects (Age Category or Age) for each var. or gene m, p combination

Var	Effect	Age Cat	Trt	_Age Cat	Trt	Estimate	Std Err	DF	T Value	Raw p	Adjustment	Adj p
T10m	Trt		G1		G2	0.572	0.322	60	1.78	0.0810	Tukey-K	0.39745
T10m	Trt		G1		G3	0.842	0.322	60	2.61	0.011349	Tukey-K	0.08084
T10m	Trt		G1		G4	0.836	0.322	60	2.59	0.011933	Tukey-K	0.084500
T10m	Trt		G1		G5	0.455	0.322	60	1.41	0.163229	Tukey-K	0.62272
T10m	Trt		G2		G3	0.27	0.322	60	0.84	0.405801	Tukey-K	0.917920
T10m	Trt		G2		G4	0.264	0.322	60	0.82	0.416585	Tukey-K	0.92407
T10m	Trt		G2		G5	-0.12	0.322	60	-0.36	0.717561	Tukey-K	0.99617
T10m	Trt		G3		G4	-0.01	0.322	60	-0.02	0.984756	Tukey-K	1
T10m	Trt		G3		G5	-0.39	0.322	60	-1.2	0.234613	Tukey-K	0.75098
T10m	Trt		G4		G5	-0.38	0.322	60	-1.18	0.242087	Tukey-K	0.761850
T10m	AgeCat	Middle		Old		-0.19	1.129	15	-0.17	0.869420	Tukey-K	0.98472
T10m	AgeCat	Middle		Young		-0.7	1.19	15	-0.59	0.562324	Tukey-K	0.82617
T10m	AgeCat	Old		Young		-0.52	0.995	15	-0.52	0.611619	Tukey-K	0.86353
T10p	Trt		G1		G2	-0.13	0.376	59	-0.34	0.737551	Tukey-K	0.99715
T10p	Trt		G1		G3	0.279	0.376	59	0.74	0.461161	Tukey-K	0.9457
T10p	Trt		G1		G4	0.305	0.376	59	0.81	0.421321	Tukey-K	0.92661
T10p	Trt		G1		G5	0.356	0.376	59	0.95	0.347379	Tukey-K	0.877100
T10p	Trt		G2		G3	0.406	0.373	59	1.09	0.280487	Tukey-K	0.81136
T10p	Trt		G2		G4	0.431	0.373	59	1.16	0.251574	Tukey-K	0.77492
T10p	Trt		G2		G5	0.483	0.373	59	1.3	0.199776	Tukey-K	0.69422
T10p	Trt		G3		G4	0.026	0.373	59	0.07	0.945481	Tukey-K	0.99999
T10p	Trt		G3		G5	0.077	0.373	59	0.21	0.836327	Tukey-K	0.99957
T10p	Trt		G4		G5	0.052	0.373	59	0.14	0.890054	Tukey-K	0.99991
T10p	AgeCat	Middle		Old		-0.13	0.741	15	-0.17	0.866930	Tukey-K	0.98412
T10p	AgeCat	Middle		Young		1.024	0.78	15	1.31	0.209353	Tukey-K	0.410400
T10p	AgeCat	Old		Young		1.15	0.654	15	1.76	0.098930	Tukey-K	0.21671
T1m	Trt		G1		G2	-0.08	0.275	60	-0.28	0.781454	Tukey-K	0.99864
T1m	Trt		G1		G3	0.238	0.275	60	0.87	0.389099	Tukey-K	0.9076
T1m	Trt		G1		G4	0.193	0.275	60	0.7	0.485866	Tukey-K	0.95543
T1m	Trt		G1		G5	-0.18	0.275	60	-0.65	0.518508	Tukey-K	0.966090
T1m	Trt		G2		G3	0.315	0.275	60	1.15	0.256255	Tukey-K	0.78132
T1m	Trt		G2		G4	0.269	0.275	60	0.98	0.331066	Tukey-K	0.86325
T1m	Trt		G2		G5	-0.1	0.275	60	-0.37	0.712089	Tukey-K	0.99586
T1m	Trt		G3		G4	-0.05	0.275	60	-0.17	0.868472	Tukey-K	0.99982
T1m	Trt		G3		G5	-0.42	0.275	60	-1.52	0.134512	Tukey-K	0.5557
T1m	Trt		G4		G5	-0.37	0.275	60	-1.35	0.181859	Tukey-K	0.66097

T1m	AgeCat	Middle	Old		0.398	0.5	15	0.8	0.437959	Tukey-K	0.71057
T1m	AgeCat	Middle	Young		-0.19	0.527	15	-0.35	0.727698	Tukey-Kr	0.93328
T1m	AgeCat	Old	Young		-0.59	0.441	15	-1.33	0.204162	Tukey-K	0.40228
T1p	Trt		G1	G2	0.472	0.304	59	1.55	0.125662	Tukey-K	0.53264
T1p	Trt		G1	G3	0.429	0.304	59	1.41	0.162536	Tukey-K	0.62105
T1p	Trt		G1	G4	0.468	0.304	59	1.54	0.128314	Tukey-K	0.53961
T1p	Trt		G1	G5	0.523	0.304	59	1.72	0.089969	Tukey-K	0.42756
T1p	Trt		G2	G3	-0.04	0.298	59	-0.14	0.887715	Tukey-K	0.99991
T1p	Trt		G2	G4	-0	0.298	59	-0.01	0.991082	Tukey-K	1
T1p	Trt		G2	G5	0.052	0.298	59	0.17	0.862754	Tukey-K	0.99979
T1p	Trt		G3	G4	0.039	0.298	59	0.13	0.896550	Tukey-K	0.99993
T1p	Trt		G3	G5	0.094	0.298	59	0.32	0.753547	Tukey-K	0.99779
T1p	Trt		G4	G5	0.055	0.298	59	0.18	0.853980	Tukey-K	0.99973
T1p	AgeCat	Middle	Old		0.006	0.794	15	0.01	0.994092	Tukey-K	0.99997
T1p	AgeCat	Middle	Young		-0.83	0.837	15	-0.99	0.337338	Tukey-K	0.59325
T1p	AgeCat	Old	Young		-0.84	0.701	15	-1.19	0.251542	Tukey-K	0.47521
T2m	Trt		G1	G2	-0.48	0.299	60	-1.6	0.115789	Tukey-K	0.5059
T2m	Trt		G1	G3	0.106	0.299	60	0.36	0.722955	Tukey-K	0.99646
T2m	Trt		G1	G4	0.137	0.299	60	0.46	0.649065	Tukey-K	0.99076
T2m	Trt		G1	G5	-0.33	0.299	60	-1.12	0.268690	Tukey-K	0.79727
T2m	Trt		G2	G3	0.583	0.299	60	1.95	0.055613	Tukey-K	0.30195
T2m	Trt		G2	G4	0.613	0.299	60	2.05	0.044425	Tukey-K	0.25402
T2m	Trt		G2	G5	0.143	0.299	60	0.48	0.633420	Tukey-K	0.98896
T2m	Trt		G3	G4	0.03	0.299	60	0.1	0.919746	Tukey-K	0.99998
T2m	Trt		G3	G5	-0.44	0.299	60	-1.47	0.146083	Tukey-K	0.58397
T2m	Trt		G4	G5	-0.47	0.299	60	-1.57	0.120796	Tukey-K	0.51974
T2m	AgeCat	Middle	Old		0.435	0.442	15	0.98	0.340937	Tukey-K	0.598
T2m	AgeCat	Middle	Young		-0.52	0.466	15	-1.12	0.278433	Tukey-K	0.51427
T2m	AgeCat	Old	Young		-0.96	0.39	15	-2.46	0.026547	Tukey-K	0.06475
T2p	Trt		G1	G2	0.227	0.313	60	0.73	0.469621	Tukey-K	0.94922
T2p	Trt		G1	G3	0.083	0.313	60	0.26	0.791993	Tukey-K	0.99889
T2p	Trt		G1	G4	0.128	0.313	60	0.41	0.684460	Tukey-K	0.994
T2p	Trt		G1	G5	0.249	0.313	60	0.8	0.428585	Tukey-K	0.93048
T2p	Trt		G2	G3	-0.14	0.313	60	-0.46	0.645176	Tukey-K	0.99034
T2p	Trt		G2	G4	-0.1	0.313	60	-0.32	0.750574	Tukey-K	0.99769
T2p	Trt		G2	G5	0.022	0.313	60	0.07	0.944977	Tukey-K	0.99999
T2p	Trt		G3	G4	0.045	0.313	60	0.14	0.886405	Tukey-K	0.9999
T2p	Trt		G3	G5	0.166	0.313	60	0.53	0.596612	Tukey-K	0.98367
T2p	Trt		G4	G5	0.121	0.313	60	0.39	0.698910	Tukey-K	0.99504
T2p	AgeCat	Middle	Old		-0.6	0.828	15	-0.73	0.477318	Tukey-K	0.75054
T2p	AgeCat	Middle	Young		-1.36	0.873	15	-1.55	0.140961	Tukey-K	0.29505

T2p	AgeCat	Old	Young		-0.75	0.73	15	-1.03	0.318755	Tukey-K	0.56933
T3m	Trt	G1	G2		-0.14	0.309	60	-0.44	0.662185	Tukey-K	0.99209
T3m	Trt	G1	G3		0.133	0.309	60	0.43	0.669045	Tukey-K	0.99272
T3m	Trt	G1	G4		0.333	0.309	60	1.08	0.285805	Tukey-K	0.81758
T3m	Trt	G1	G5		-0.04	0.309	60	-0.14	0.892665	Tukey-K	0.99992
T3m	Trt	G2	G3		0.268	0.309	60	0.87	0.388501	Tukey-K	0.90721
T3m	Trt	G2	G4		0.469	0.309	60	1.52	0.134759	Tukey-K	0.55632
T3m	Trt	G2	G5		0.094	0.309	60	0.3	0.762508	Tukey-K	0.9981
T3m	Trt	G3	G4		0.2	0.309	60	0.65	0.519844	Tukey-K	0.96648
T3m	Trt	G3	G5		-0.17	0.309	60	-0.57	0.574126	Tukey-K	0.97959
T3m	Trt	G4	G5		-0.37	0.309	60	-1.21	0.230082	Tukey-K	0.74419
T3m	AgeCat	Middle	Old		0.588	0.678	15	0.87	0.399630	Tukey-K	0.66853
T3m	AgeCat	Middle	Young		-0.25	0.715	15	-0.36	0.726919	Tukey-K	0.93289
T3m	AgeCat	Old	Young		-0.84	0.598	15	-1.41	0.179424	Tukey-K	0.36171
T3p	Trt	G1	G2		0.08	0.28	60	0.29	0.775918	Tukey-K	0.9985
T3p	Trt	G1	G3		0.414	0.28	60	1.48	0.144704	Tukey-K	0.5807
T3p	Trt	G1	G4		0.403	0.28	60	1.44	0.155200	Tukey-K	0.60502
<b>T3p</b>	<b>Trt</b>	<b>G1</b>	<b>G5</b>		<b>0.537</b>	<b>0.28</b>	<b>60</b>	<b>1.92</b>	<b>0.059787</b>	Tukey-K	0.31884
T3p	Trt	G2	G3		0.334	0.28	60	1.19	0.238022	Tukey-K	0.75599
T3p	Trt	G2	G4		0.323	0.28	60	1.15	0.253242	Tukey-K	0.7773
T3p	Trt	G2	G5		0.457	0.28	60	1.63	0.107760	Tukey-K	0.48286
T3p	Trt	G3	G4		-0.01	0.28	60	-0.04	0.969629	Tukey-K	1
T3p	Trt	G3	G5		0.124	0.28	60	0.44	0.660855	Tukey-K	0.99196
T3p	Trt	G4	G5		0.134	0.28	60	0.48	0.633571	Tukey-K	0.98898
T3p	AgeCat	Middle	Old		-0.12	0.803	15	-0.15	0.885279	Tukey-K	0.98821
T3p	AgeCat	Middle	Young		-0.57	0.846	15	-0.67	0.513474	Tukey-K	0.78447
T3p	AgeCat	Old	Young		-0.45	0.708	15	-0.63	0.535908	Tukey-K	0.8042
T4m	Trt	G1	G2		0.143	0.267	60	0.53	0.594943	Tukey-K	0.98339
T4m	Trt	G1	G3		0.117	0.267	60	0.44	0.663864	Tukey-K	0.99225
T4m	Trt	G1	G4		0.13	0.267	60	0.49	0.628482	Tukey-K	0.98834
T4m	Trt	G1	G5		-0.12	0.267	60	-0.44	0.661917	Tukey-K	0.99206
T4m	Trt	G2	G3		-0.03	0.267	60	-0.1	0.922421	Tukey-K	0.99998
T4m	Trt	G2	G4		-0.01	0.267	60	-0.05	0.961735	Tukey-K	1
T4m	Trt	G2	G5		-0.26	0.267	60	-0.97	0.333974	Tukey-K	0.86582
T4m	Trt	G3	G4		0.013	0.267	60	0.05	0.960592	Tukey-K	1
T4m	Trt	G3	G5		-0.23	0.267	60	-0.88	0.384420	Tukey-K	0.90453
T4m	Trt	G4	G5		-0.25	0.267	60	-0.93	0.358254	Tukey-K	0.8858
T4m	AgeCat	Middle	Old		-0.04	0.483	15	-0.09	0.927602	Tukey-K	0.99531
T4m	AgeCat	Middle	Young		-0.28	0.509	15	-0.56	0.586162	Tukey-K	0.84482
T4m	AgeCat	Old	Young		-0.24	0.426	15	-0.56	0.583602	Tukey-K	0.84287
T4p	Trt	G1	G2		0.073	0.393	60	0.19	0.852386	Tukey-K	0.99972

T4p	Trt		G1	G3	0.002	0.393	60	0.01	0.995816	Tukey-K	1
T4p	Trt		G1	G4	0.038	0.393	60	0.1	0.924074	Tukey-K	0.99998
T4p	Trt		G1	G5	0.107	0.393	60	0.27	0.785719	Tukey-K	0.99875
T4p	Trt		G2	G3	-0.07	0.393	60	-0.18	0.856498	Tukey-K	0.99975
T4p	Trt		G2	G4	-0.04	0.393	60	-0.09	0.927658	Tukey-K	0.99998
T4p	Trt		G2	G5	0.034	0.393	60	0.09	0.931583	Tukey-K	0.99999
T4p	Trt		G3	G4	0.036	0.393	60	0.09	0.928239	Tukey-K	0.99998
T4p	Trt		G3	G5	0.105	0.393	60	0.27	0.789750	Tukey-K	0.99884
T4p	Trt		G4	G5	0.07	0.393	60	0.18	0.859801	Tukey-K	0.99977
T4p	AgeCat	Middle		Old	-0.14	0.712	15	-0.19	0.851429	Tukey-K	0.98021
T4p	AgeCat	Middle		Young	-0.87	0.75	15	-1.17	0.261616	Tukey-K	0.49014
T4p	AgeCat	Old		Young	-0.74	0.628	15	-1.18	0.257075	Tukey-K	0.48351
T5m	Trt		G1	G2	0.059	0.307	48	0.19	0.847295	Tukey-K	0.99967
T5m	Trt		G1	G3	0.205	0.307	48	0.67	0.507572	Tukey-K	0.96238
T5m	Trt		G1	G4	0.065	0.307	48	0.21	0.834144	Tukey-K	0.99955
T5m	Trt		G1	G5	-0.17	0.307	48	-0.54	0.590117	Tukey-K	0.98237
T5m	Trt		G2	G3	0.146	0.307	48	0.47	0.637643	Tukey-K	0.98935
T5m	Trt		G2	G4	0.005	0.307	48	0.02	0.986572	Tukey-K	1
T5m	Trt		G2	G5	-0.23	0.307	48	-0.74	0.465361	Tukey-K	0.94697
T5m	Trt		G3	G4	-0.14	0.307	48	-0.46	0.649668	Tukey-K	0.99072
T5m	Trt		G3	G5	-0.37	0.307	48	-1.21	0.232231	Tukey-K	0.74567
T5m	Trt		G4	G5	-0.23	0.307	48	-0.75	0.455224	Tukey-K	0.94261
T5m	AgeCat	Middle		Old	0.047	1.279	12	0.04	0.971283	Tukey-K	0.99926
T5m	AgeCat	Middle		Young	0.909	1.279	12	0.71	0.490964	Tukey-K	0.76204
T5m	AgeCat	Old		Young	0.862	1.045	12	0.83	0.425369	Tukey-K	0.69518
T5p	Trt		G1	G2	-0.44	0.358	48	-1.23	0.225314	Tukey-K	0.73505
T5p	Trt		G1	G3	-0.3	0.358	48	-0.84	0.405242	Tukey-K	0.91682
T5p	Trt		G1	G4	-0.02	0.358	48	-0.06	0.955590	Tukey-K	1
T5p	Trt		G1	G5	0.051	0.358	48	0.14	0.887594	Tukey-K	0.9999
T5p	Trt		G2	G3	0.139	0.358	48	0.39	0.699270	Tukey-K	0.99501
T5p	Trt		G2	G4	0.419	0.358	48	1.17	0.246845	Tukey-K	0.76687
T5p	Trt		G2	G5	0.49	0.358	48	1.37	0.176929	Tukey-K	0.64914
T5p	Trt		G3	G4	0.28	0.358	48	0.78	0.437057	Tukey-K	0.93411
T5p	Trt		G3	G5	0.351	0.358	48	0.98	0.331122	Tukey-K	0.86214
T5p	Trt		G4	G5	0.071	0.358	48	0.2	0.843817	Tukey-K	0.99964
T5p	AgeCat	Middle		Old	-0.91	1.093	12	-0.83	0.423510	Tukey-K	0.69316
T5p	AgeCat	Middle		Young	0.453	1.093	12	0.41	0.686061	Tukey-K	0.91047
T5p	AgeCat	Old		Young	1.358	0.892	12	1.52	0.153901	Tukey-K	0.31564
T6m	Trt		G1	G2	-0.63	0.519	54	-1.21	0.232174	Tukey-K	0.74658
T6m	Trt		G1	G3	-0.18	0.519	54	-0.34	0.736978	Tukey-K	0.99712
T6m	Trt		G1	G4	9E-04	0.519	54	0	0.998612	Tukey-K	1

T6m	Trt		G1	G5	-0.37	0.519	54	-0.72	0.476834	Tukey-K	0.95183
T6m	Trt		G2	G3	0.452	0.524	52	0.86	0.392591	Tukey-K	0.90937
T6m	Trt		G2	G4	0.628	0.524	52	1.2	0.236312	Tukey-K	0.75247
T6m	Trt		G2	G5	0.255	0.524	52	0.49	0.628266	Tukey-K	0.98824
T6m	Trt		G3	G4	0.176	0.524	52	0.34	0.738249	Tukey-K	0.99717
T6m	Trt		G3	G5	-0.2	0.524	52	-0.38	0.709163	Tukey-K	0.99566
T6m	Trt		G4	G5	-0.37	0.524	52	-0.71	0.480259	Tukey-K	0.9531
T6m	AgeCat	Middle		Old	-0.13	1.23	15	-0.1	0.919292	Tukey-K	0.99416
T6m	AgeCat	Middle		Young	-0.87	1.264	15	-0.69	0.499681	Tukey-K	0.77189
T6m	AgeCat	Old		Young	-0.75	1.056	14	-0.71	0.490691	Tukey-K	0.7626
T6p	Trt		G1	G2	-0.35	0.487	51	-0.72	0.475678	Tukey-K	0.95127
T6p	Trt		G1	G3	-0.01	0.479	51	-0.02	0.981468	Tukey-K	1
T6p	Trt		G1	G4	0.212	0.479	51	0.44	0.659225	Tukey-K	0.99174
T6p	Trt		G1	G5	0.437	0.479	51	0.91	0.365375	Tukey-K	0.89049
T6p	Trt		G2	G3	0.338	0.487	51	0.7	0.489836	Tukey-K	0.95654
T6p	Trt		G2	G4	0.562	0.487	51	1.16	0.253372	Tukey-K	0.77634
T6p	Trt		G2	G5	0.787	0.487	51	1.62	0.11190	Tukey-K	0.49332
T6p	Trt		G3	G4	0.224	0.479	51	0.47	0.64255	Tukey-K	0.98996
T6p	Trt		G3	G5	0.449	0.479	51	0.94	0.35335	Tukey-K	0.88125
T6p	Trt		G4	G5	0.225	0.479	51	0.47	0.640547	Tukey-K	0.98973
T6p	AgeCat	Middle		Old	-1	1.3	13	-0.77	0.455078	Tukey-K	0.72723
T6p	AgeCat	Middle		Young	-0.31	1.333	13	-0.23	0.819834	Tukey-K	0.97073
T6p	AgeCat	Old		Young	0.691	1.049	13	0.66	0.521559	Tukey-K	0.79083
T7m	Trt		G1	G2	0.25	0.465	59	0.54	0.592245	Tukey-K	0.98292
T7m	Trt		G1	G3	0.955	0.445	59	2.15	0.035895	Tukey-K	0.21437
T7m	Trt		G1	G4	0.95	0.445	59	2.14	0.036773	Tukey-K	0.21857
T7m	Trt		G1	G5	-0.07	0.445	59	-0.17	0.867202	Tukey-K	0.99982
T7m	Trt		G2	G3	0.704	0.465	59	1.51	0.135386	Tukey-K	0.5578
T7m	Trt		G2	G4	0.699	0.465	59	1.5	0.137934	Tukey-K	0.56415
T7m	Trt		G2	G5	-0.33	0.465	59	-0.7	0.487268	Tukey-K	0.95591
T7m	Trt		G3	G4	-0	0.445	59	-0.01	0.991698	Tukey-K	1
T7m	Trt		G3	G5	-1.03	0.445	59	-2.32	0.024100	Tukey-K	0.15456
T7m	Trt		G4	G5	-1.02	0.445	59	-2.3	0.024719	Tukey-K	0.15787
T7m	AgeCat	Middle		Old	0.768	1.125	15	0.68	0.505071	Tukey-K	0.77689
T7m	AgeCat	Middle		Young	-0.39	1.185	15	-0.33	0.748186	Tukey-K	0.943
T7m	AgeCat	Old		Young	-1.16	0.989	15	-1.17	0.261067	Tukey-K	0.48924
T7p	Trt		G1	G2	-0.33	0.472	60	-0.69	0.492841	Tukey-K	0.9579
T7p	Trt		G1	G3	-0.09	0.472	60	-0.2	0.841622	Tukey-K	0.99963
T7p	Trt		G1	G4	0.169	0.472	60	0.36	0.721520	Tukey-K	0.99639
T7p	Trt		G1	G5	-0.15	0.472	60	-0.32	0.751742	Tukey-K	0.99773
T7p	Trt		G2	G3	0.231	0.472	60	0.49	0.626389	Tukey-K	0.98807

T7p	Trt		G2	G4	0.495	0.472	60	1.05	0.298783	Tukey-K	0.83182
T7p	Trt		G2	G5	0.176	0.472	60	0.37	0.711038	Tukey-K	0.9958
T7p	Trt		G3	G4	0.264	0.472	60	0.56	0.578381	Tukey-K	0.98041
T7p	Trt		G3	G5	-0.06	0.472	60	-0.12	0.907164	Tukey-K	0.99996
T7p	Trt		G4	G5	-0.32	0.472	60	-0.68	0.501698	Tukey-K	0.96088
T7p	AgeCat	Middle	Old		-0	0.952	15	-0.01	0.995978	Tukey-K	0.99999
T7p	AgeCat	Middle	Young		0.469	1.004	15	0.47	0.647288	Tukey-K	0.88766
T7p	AgeCat	Old	Young		0.474	0.84	15	0.56	0.581188	Tukey-K	0.84102
T8m	Trt		G1	G2	0.091	0.52	60	0.17	0.861828	Tukey-K	0.99978
T8m	Trt		G1	G3	0.302	0.52	60	0.58	0.564126	Tukey-K	0.97754
T8m	Trt		G1	G4	0.561	0.52	60	1.08	0.285230	Tukey-K	0.81693
T8m	Trt		G1	G5	0.141	0.52	60	0.27	0.787116	Tukey-K	0.99878
T8m	Trt		G2	G3	0.211	0.52	60	0.41	0.686813	Tukey-K	0.99418
T8m	Trt		G2	G4	0.47	0.52	60	0.9	0.369888	Tukey-K	0.89446
T8m	Trt		G2	G5	0.05	0.52	60	0.1	0.923468	Tukey-K	0.99998
T8m	Trt		G3	G4	0.259	0.52	60	0.5	0.620074	Tukey-K	0.98722
T8m	Trt		G3	G5	-0.16	0.52	60	-0.31	0.758640	Tukey-K	0.99797
T8m	Trt		G4	G5	-0.42	0.52	60	-0.81	0.422854	Tukey-K	0.92748
T8m	AgeCat	Middle	Old		0.103	1.417	15	0.07	0.942989	Tukey-K	0.99709
T8m	AgeCat	Middle	Young		0.428	1.494	15	0.29	0.778224	Tukey-K	0.95582
T8m	AgeCat	Old	Young		0.325	1.25	15	0.26	0.798188	Tukey-K	0.96343
T8p	Trt		G1	G2	0.055	0.449	60	0.12	0.903675	Tukey-K	0.99995
T8p	Trt		G1	G3	-0.81	0.449	60	-1.8	0.077519	Tukey-K	0.3854
T8p	Trt		G1	G4	0.211	0.449	60	0.47	0.640677	Tukey-K	0.98983
T8p	Trt		G1	G5	0.215	0.449	60	0.48	0.633719	Tukey-K	0.989
T8p	Trt		G2	G3	-0.86	0.449	60	-1.92	0.059926	Tukey-K	0.3194
T8p	Trt		G2	G4	0.156	0.449	60	0.35	0.729360	Tukey-K	0.99678
T8p	Trt		G2	G5	0.16	0.449	60	0.36	0.722038	Tukey-K	0.99641
T8p	Trt		G3	G4	1.016	0.449	60	2.27	0.027124	Tukey-K	0.17066
T8p	Trt		G3	G5	1.021	0.449	60	2.28	0.026492	Tukey-K	0.16736
T8p	Trt		G4	G5	0.004	0.449	60	0.01	0.992202	Tukey-K	1
T8p	AgeCat	Middle	Old		-0.25	0.968	15	-0.26	0.798855	Tukey-K	0.96368
T8p	AgeCat	Middle	Young		1.866	1.021	15	1.83	0.087486	Tukey-K	0.19428
T8p	AgeCat	Old	Young		2.117	0.854	15	2.48	0.025534	Tukey-Kr	0.06242
T9m	Trt		G1	G2	0.069	0.34	60	0.2	0.839533	Tukey-K	0.99961
T9m	Trt		G1	G3	0.188	0.34	60	0.55	0.583324	Tukey-K	0.98134
T9m	Trt		G1	G4	0.451	0.34	60	1.33	0.189721	Tukey-K	0.67601
T9m	Trt		G1	G5	-0.24	0.34	60	-0.71	0.482967	Tukey-K	0.95437
T9m	Trt		G2	G3	0.118	0.34	60	0.35	0.728945	Tukey-K	0.99676
T9m	Trt		G2	G4	0.382	0.34	60	1.12	0.265882	Tukey-K	0.79376
T9m	Trt		G2	G5	-0.31	0.34	60	-0.91	0.366832	Tukey-K	0.89224

T9m	Trt		G3	G4	0.264	0.34	60	0.77	0.441431	Tukey-K	0.93686
T9m	Trt		G3	G5	-0.43	0.34	60	-1.26	0.213464	Tukey-K	0.71784
T9m	Trt		G4	G5	-0.69	0.34	60	-2.03	0.046550	Tukey-K	0.26344
T9m	AgeCat	Middle	Old		0.661	1.065	15	0.62	0.544108	Tukey-K	0.81117
T9m	AgeCat	Middle	Young		0.969	1.123	15	0.86	0.401642	Tukey-K	0.67082
T9m	AgeCat	Old	Young		0.308	0.939	15	0.33	0.747554	Tukey-K	0.94269
T9p	Trt		G1	G2	-0.41	0.467	60	-0.87	0.387282	Tukey-K	0.90642
T9p	Trt		G1	G3	-0.47	0.467	60	-1.01	0.315047	Tukey-K	0.84833
T9p	Trt		G1	G4	0.193	0.467	60	0.41	0.679909	Tukey-K	0.99365
T9p	Trt		G1	G5	0.043	0.467	60	0.09	0.926653	Tukey-K	0.99998
T9p	Trt		G2	G3	-0.07	0.467	60	-0.14	0.887340	Tukey-K	0.99991
T9p	Trt		G2	G4	0.6	0.467	60	1.29	0.203556	Tukey-K	0.70102
T9p	Trt		G2	G5	0.449	0.467	60	0.96	0.339243	Tukey-K	0.87038
T9p	Trt		G3	G4	0.666	0.467	60	1.43	0.158541	Tukey-K	0.61248
T9p	Trt		G3	G5	0.516	0.467	60	1.11	0.273307	Tukey-K	0.80293
T9p	Trt		G4	G5	-0.15	0.467	60	-0.32	0.748450	Tukey-K	0.9976
T9p	AgeCat	Middle	Old		-0.02	1.172	15	-0.02	0.985840	Tukey-K	0.99982
T9p	AgeCat	Middle	Young		2.197	1.235	15	1.78	0.095494	Tukey-K	0.21003
T9p	AgeCat	Old	Young		2.218	1.033	15	2.15	0.048563	Tukey-K	0.11378

TEST 3: gave slice comparisons or interaction comparisons.

Var	StmtNo	Effect	Slice	Trt	_Trt	Estimate	StdErr	DF	tValue	Raw p	Adjustment	Adj p
T10m	1	AgeCat*Trt	Middle	G1	G2	-0	0.657	60	-0.04	0.96857	Tukey-K	1
T10m	1	AgeCat*Trt	Middle	G1	G3	0.13	0.657	60	0.199	0.84284	Tukey-K	0.9996
T10m	1	AgeCat*Trt	Middle	G1	G4	0.62	0.657	60	0.947	0.34752	Tukey-K	0.8773
T10m	1	AgeCat*Trt	Middle	G1	G5	-0.2	0.657	60	-0.38	0.70739	Tukey-K	0.9956
T10m	1	AgeCat*Trt	Middle	G2	G3	0.16	0.657	60	0.239	0.81216	Tukey-K	0.9993
T10m	1	AgeCat*Trt	Middle	G2	G4	0.65	0.657	60	0.986	0.3279	Tukey-K	0.8604
T10m	1	AgeCat*Trt	Middle	G2	G5	-0.2	0.657	60	-0.34	0.73685	Tukey-K	0.9971
T10m	1	AgeCat*Trt	Middle	G3	G4	0.49	0.657	60	0.748	0.45756	Tukey-K	0.9442
T10m	1	AgeCat*Trt	Middle	G3	G5	-0.4	0.657	60	-0.58	0.56659	Tukey-K	0.9781
T10m	1	AgeCat*Trt	Middle	G4	G5	-0.9	0.657	60	-1.32	0.19053	Tukey-K	0.6775
T10m	1	AgeCat*Trt	Old	G1	G2	1.33	0.465	60	2.866	0.00572	Tukey-K	0.04368
T10m	1	AgeCat*Trt	Old	G1	G3	1.6	0.465	60	3.449	0.00103	Tukey-K	0.00883
T10m	1	AgeCat*Trt	Old	G1	G4	1.41	0.465	60	3.026	0.00364	Tukey-K	0.02886
T10m	1	AgeCat*Trt	Old	G1	G5	1.31	0.465	60	2.824	0.00644	Tukey-K	0.04864
T10m	1	AgeCat*Trt	Old	G2	G3	0.27	0.465	60	0.583	0.562218	Tukey-K	0.9771
T10m	1	AgeCat*Trt	Old	G2	G4	0.07	0.465	60	0.16	0.873483	Tukey-K	0.9998
T10m	1	AgeCat*Trt	Old	G2	G5	-0	0.465	60	-0.04	0.965929	Tukey-K	1
T10m	1	AgeCat*Trt	Old	G3	G4	-0.2	0.465	60	-0.42	0.673900	Tukey-K	0.9931
T10m	1	AgeCat*Trt	Old	G3	G5	-0.3	0.465	60	-0.63	0.533895	Tukey-K	0.9704
T10m	1	AgeCat*Trt	Old	G4	G5	-0.1	0.465	60	-0.2	0.839969	Tukey-K	0.9996
T10m	1	AgeCat*Trt	Young	G1	G2	0.41	0.536	60	0.766	0.446630	Tukey-K	0.9393
T10m	1	AgeCat*Trt	Young	G1	G3	0.79	0.536	60	1.478	0.144550	Tukey-K	0.5803
T10m	1	AgeCat*Trt	Young	G1	G4	0.48	0.536	60	0.894	0.374781	Tukey-K	0.8979
T10m	1	AgeCat*Trt	Young	G1	G5	0.3	0.536	60	0.562	0.576543	Tukey-K	0.9801
T10m	1	AgeCat*Trt	Young	G2	G3	0.38	0.536	60	0.712	0.479072	Tukey-K	0.9529
T10m	1	AgeCat*Trt	Young	G2	G4	0.07	0.536	60	0.128	0.898475	Tukey-K	0.9999
T10m	1	AgeCat*Trt	Young	G2	G5	-0.1	0.536	60	-0.2	0.838596	Tukey-K	0.9996
T10m	1	AgeCat*Trt	Young	G3	G4	-0.3	0.536	60	-0.58	0.561330	Tukey-K	0.9769
T10m	1	AgeCat*Trt	Young	G3	G5	-0.5	0.536	60	-0.92	0.362905	Tukey-K	0.8893
T10m	1	AgeCat*Trt	Young	G4	G5	-0.2	0.536	60	-0.33	0.740516	Tukey-K	0.9973
T10p	1	AgeCat*Trt	Middle	G1	G2	-0.3	0.759	59	-0.42	0.678960	Tukey-Kr	0.9936
T10p	1	AgeCat*Trt	Middle	G1	G3	0.29	0.759	59	0.382	0.704047	Tukey-K	0.9954
T10p	1	AgeCat*Trt	Middle	G1	G4	-0.3	0.759	59	-0.37	0.713049	Tukey-K	0.9959
T10p	1	AgeCat*Trt	Middle	G1	G5	0.38	0.759	59	0.504	0.615871	Tukey-K	0.9866
T10p	1	AgeCat*Trt	Middle	G2	G3	0.61	0.759	59	0.798	0.428271	Tukey-K	0.9303
T10p	1	AgeCat*Trt	Middle	G2	G4	0.04	0.759	59	0.046	0.963143	Tukey-K	1
T10p	1	AgeCat*Trt	Middle	G2	G5	0.7	0.759	59	0.92	0.36115	Tukey-K	0.8879
T10p	1	AgeCat*Trt	Middle	G3	G4	-0.6	0.759	59	-0.75	0.45549	Tukey-K	0.9433

T10p	1	AgeCat*Trt	Middle	G3	G5	0.09	0.759	59	0.123	0.90278	Tukey-K	0.9999
T10p	1	AgeCat*Trt	Middle	G4	G5	0.66	0.759	59	0.874	0.38571	Tukey-K	0.9053
T10p	1	AgeCat*Trt	Old	G1	G2	-0.3	0.56	59	-0.59	0.55472	Tukey-K	0.9755
T10p	1	AgeCat*Trt	Old	G1	G3	-0.1	0.56	59	-0.2	0.83905	Tukey-K	0.9996
T10p	1	AgeCat*Trt	Old	G1	G4	0.54	0.56	59	0.97	0.33613	Tukey-K	0.8676
T10p	1	AgeCat*Trt	Old	G1	G5	-0.2	0.56	59	-0.32	0.75294	Tukey-K	0.9978
T10p	1	AgeCat*Trt	Old	G2	G3	0.22	0.537	59	0.407	0.68571	Tukey-K	0.9941
T10p	1	AgeCat*Trt	Old	G2	G4	0.88	0.537	59	1.63	0.10836	Tukey-K	0.4844
T10p	1	AgeCat*Trt	Old	G2	G5	0.16	0.537	59	0.29	0.77308	Tukey-K	0.9984
T10p	1	AgeCat*Trt	Old	G3	G4	0.66	0.537	59	1.224	0.22594	Tukey-K	0.7377
T10p	1	AgeCat*Trt	Old	G3	G5	-0.1	0.537	59	-0.12	0.90726	Tukey-K	1
T10p	1	AgeCat*Trt	Old	G4	G5	-0.7	0.537	59	-1.34	0.18517	Tukey-K	0.6672
T10p	1	AgeCat*Trt	Young	G1	G2	0.27	0.62	59	0.433	0.66674	Tukey-K	0.9925
T10p	1	AgeCat*Trt	Young	G1	G3	0.66	0.62	59	1.067	0.29019	Tukey-K	0.8224
T10p	1	AgeCat*Trt	Young	G1	G4	0.65	0.62	59	1.051	0.29734	Tukey-K	0.8302
T10p	1	AgeCat*Trt	Young	G1	G5	0.86	0.62	59	1.392	0.16901	Tukey-K	0.6348
T10p	1	AgeCat*Trt	Young	G2	G3	0.39	0.62	59	0.635	0.5282	Tukey-K	0.9688
T10p	1	AgeCat*Trt	Young	G2	G4	0.38	0.62	59	0.619	0.53851	Tukey-K	0.9715
T10p	1	AgeCat*Trt	Young	G2	G5	0.59	0.62	59	0.96	0.34113	Tukey-K	0.8719
T10p	1	AgeCat*Trt	Young	G3	G4	-0	0.62	59	-0.02	0.98742	Tukey-K	1
T10p	1	AgeCat*Trt	Young	G3	G5	0.2	0.62	59	0.325	0.7462	Tukey-K	0.9975
T10p	1	AgeCat*Trt	Young	G4	G5	0.21	0.62	59	0.341	0.7343	Tukey-K	0.997
T1m	1	AgeCat*Trt	Middle	G1	G2	-0.1	0.56	60	-0.24	0.81252	Tukey-K	0.9993
T1m	1	AgeCat*Trt	Middle	G1	G3	0.18	0.56	60	0.314	0.75459	Tukey-K	0.9978
T1m	1	AgeCat*Trt	Middle	G1	G4	0.41	0.56	60	0.733	0.46626	Tukey-K	0.9479
T1m	1	AgeCat*Trt	Middle	G1	G5	-0.3	0.56	60	-0.61	0.54246	Tukey-K	0.9726
T1m	1	AgeCat*Trt	Middle	G2	G3	0.31	0.56	60	0.552	0.58282	Tukey-K	0.9812
T1m	1	AgeCat*Trt	Middle	G2	G4	0.54	0.56	60	0.971	0.33521	Tukey-K	0.8669
T1m	1	AgeCat*Trt	Middle	G2	G5	-0.2	0.56	60	-0.37	0.70946	Tukey-K	0.9957
T1m	1	AgeCat*Trt	Middle	G3	G4	0.23	0.56	60	0.419	0.67655	Tukey-K	0.9934
T1m	1	AgeCat*Trt	Middle	G3	G5	-0.5	0.56	60	-0.93	0.35784	Tukey-K	0.8855
T1m	1	AgeCat*Trt	Middle	G4	G5	-0.8	0.56	60	-1.35	0.18342	Tukey-K	0.664
T1m	1	AgeCat*Trt	Old	G1	G2	0.15	0.396	60	0.382	0.70399	Tukey-K	0.9954
T1m	1	AgeCat*Trt	Old	G1	G3	0.3	0.396	60	0.758	0.4514	Tukey-K	0.9415
T1m	1	AgeCat*Trt	Old	G1	G4	0.72	0.396	60	1.808	0.07568	Tukey-K	0.3789
T1m	1	AgeCat*Trt	Old	G1	G5	-0.1	0.396	60	-0.18	0.85653	Tukey-K	0.9997
T1m	1	AgeCat*Trt	Old	G2	G3	0.15	0.396	60	0.376	0.70803	Tukey-K	0.9956
T1m	1	AgeCat*Trt	Old	G2	G4	0.56	0.396	60	1.426	0.1591	Tukey-K	0.6137
T1m	1	AgeCat*Trt	Old	G2	G5	-0.2	0.396	60	-0.56	0.57531	Tukey-K	0.9798
T1m	1	AgeCat*Trt	Old	G3	G4	0.42	0.396	60	1.05	0.29814	Tukey-K	0.8311
T1m	1	AgeCat*Trt	Old	G3	G5	-0.4	0.396	60	-0.94	0.35118	Tukey-K	0.8802

T1m	1	AgeCat*Trt	Old	G4	G5	-0.8	0.396	60	-1.99	0.05125	Tukey-K	0.2837
T1m	1	AgeCat*Trt	Young	G1	G2	-0.2	0.457	60	-0.54	0.59036	Tukey-K	0.9826
T1m	1	AgeCat*Trt	Young	G1	G3	0.24	0.457	60	0.523	0.60296	Tukey-K	0.9847
T1m	1	AgeCat*Trt	Young	G1	G4	-0.5	0.457	60	-1.2	0.23513	Tukey-K	0.7518
T1m	1	AgeCat*Trt	Young	G1	G5	-0.1	0.457	60	-0.26	0.79318	Tukey-K	0.9989
T1m	1	AgeCat*Trt	Young	G2	G3	0.49	0.457	60	1.064	0.29153	Tukey-K	0.824
T1m	1	AgeCat*Trt	Young	G2	G4	-0.3	0.457	60	-0.66	0.51302	Tukey-K	0.9645
T1m	1	AgeCat*Trt	Young	G2	G5	0.13	0.457	60	0.278	0.78206	Tukey-K	0.9987
T1m	1	AgeCat*Trt	Young	G3	G4	-0.8	0.457	60	-1.72	0.09019	Tukey-K	0.4284
T1m	1	AgeCat*Trt	Young	G3	G5	-0.4	0.457	60	-0.79	0.43481	Tukey-K	0.9336
T1m	1	AgeCat*Trt	Young	G4	G5	0.43	0.457	60	0.936	0.35306	Tukey-K	0.8817
T1p	1	AgeCat*Trt	Middle	G1	G2	0.71	0.607	59	1.176	0.24429	Tukey-K	0.7648
T1p	1	AgeCat*Trt	Middle	G1	G3	1.05	0.607	59	1.731	0.0887	Tukey-K	0.4234
T1p	1	AgeCat*Trt	Middle	G1	G4	0.67	0.607	59	1.096	0.27745	Tukey-K	0.8078
T1p	1	AgeCat*Trt	Middle	G1	G5	1.02	0.607	59	1.673	0.0996	Tukey-K	0.4581
T1p	1	AgeCat*Trt	Middle	G2	G3	0.34	0.607	59	0.555	0.58107	Tukey-K	0.9809
T1p	1	AgeCat*Trt	Middle	G2	G4	-0	0.607	59	-0.08	0.9366	Tukey-K	1
T1p	1	AgeCat*Trt	Middle	G2	G5	0.3	0.607	59	0.497	0.62096	Tukey-K	0.9873
T1p	1	AgeCat*Trt	Middle	G3	G4	-0.4	0.607	59	-0.63	0.52804	Tukey-K	0.9688
T1p	1	AgeCat*Trt	Middle	G3	G5	-0	0.607	59	-0.06	0.95413	Tukey-K	1
T1p	1	AgeCat*Trt	Middle	G4	G5	0.35	0.607	59	0.577	0.56614	Tukey-K	0.9779
T1p	1	AgeCat*Trt	Old	G1	G2	0.29	0.429	59	0.683	0.49738	Tukey-K	0.9594
T1p	1	AgeCat*Trt	Old	G1	G3	0	0.429	59	0.003	0.9976	Tukey-K	1
T1p	1	AgeCat*Trt	Old	G1	G4	0.65	0.429	59	1.516	0.13501	Tukey-K	0.5568
T1p	1	AgeCat*Trt	Old	G1	G5	0.18	0.429	59	0.427	0.67118	Tukey-K	0.9929
T1p	1	AgeCat*Trt	Old	G2	G3	-0.3	0.429	59	-0.68	0.49928	Tukey-K	0.9601
T1p	1	AgeCat*Trt	Old	G2	G4	0.36	0.429	59	0.833	0.40842	Tukey-K	0.9194
T1p	1	AgeCat*Trt	Old	G2	G5	-0.1	0.429	59	-0.26	0.79869	Tukey-K	0.999
T1p	1	AgeCat*Trt	Old	G3	G4	0.65	0.429	59	1.512	0.13578	Tukey-K	0.5587
T1p	1	AgeCat*Trt	Old	G3	G5	0.18	0.429	59	0.424	0.67338	Tukey-K	0.9931
T1p	1	AgeCat*Trt	Old	G4	G5	-0.5	0.429	59	-1.09	0.28067	Tukey-K	0.8115
T1p	1	AgeCat*Trt	Young	G1	G2	0.41	0.526	59	0.775	0.44139	Tukey-K	0.9368
T1p	1	AgeCat*Trt	Young	G1	G3	0.24	0.526	59	0.448	0.65569	Tukey-K	0.9914
T1p	1	AgeCat*Trt	Young	G1	G4	0.09	0.526	59	0.168	0.86724	Tukey-K	0.9998
T1p	1	AgeCat*Trt	Young	G1	G5	0.37	0.526	59	0.705	0.4834	Tukey-K	0.9545
T1p	1	AgeCat*Trt	Young	G2	G3	-0.2	0.496	59	-0.35	0.73021	Tukey-K	0.9968
T1p	1	AgeCat*Trt	Young	G2	G4	-0.3	0.496	59	-0.64	0.52241	Tukey-K	0.9672
T1p	1	AgeCat*Trt	Young	G2	G5	-0	0.496	59	-0.07	0.94129	Tukey-K	1
T1p	1	AgeCat*Trt	Young	G3	G4	-0.1	0.496	59	-0.3	0.76752	Tukey-K	0.9983
T1p	1	AgeCat*Trt	Young	G3	G5	0.14	0.496	59	0.273	0.78616	Tukey-K	0.9988
T1p	1	AgeCat*Trt	Young	G4	G5	0.28	0.496	59	0.57	0.57116	Tukey-K	0.979

T2m	1	AgeCat*Trt	Middle	G1	G2	-0.3	0.608	60	-0.45	0.65229	Tukey-K	0.9911
T2m	1	AgeCat*Trt	Middle	G1	G3	0.8	0.608	60	1.312	0.19467	Tukey-K	0.6852
T2m	1	AgeCat*Trt	Middle	G1	G4	0.6	0.608	60	0.991	0.3256	Tukey-K	0.8583
T2m	1	AgeCat*Trt	Middle	G1	G5	Var	0.608	60	0.185	0.85376	Tukey-K	0.9997
T2m	1	AgeCat*Trt	Middle	G2	G3	1.07	0.608	60	1.764	0.08276	Tukey-K	0.4036
T2m	1	AgeCat*Trt	Middle	G2	G4	0.88	0.608	60	1.444	0.15395	Tukey-K	0.6022
T2m	1	AgeCat*Trt	Middle	G2	G5	0.39	0.608	60	0.638	0.52592	Tukey-K	0.9682
T2m	1	AgeCat*Trt	Middle	G3	G4	-0.2	0.608	60	-0.32	0.74977	Tukey-K	0.9977
T2m	1	AgeCat*Trt	Middle	G3	G5	-0.7	0.608	60	-1.13	0.26448	Tukey-K	0.792
T2m	1	AgeCat*Trt	Middle	G4	G5	-0.5	0.608	60	-0.81	0.42342	Tukey-K	0.9278
T2m	1	AgeCat*Trt	Old	G1	G2	-0.2	0.43	60	-0.36	0.7205	Tukey-K	0.9963
T2m	1	AgeCat*Trt	Old	G1	G3	-0.1	0.43	60	-0.29	0.77052	Tukey-K	0.9983
T2m	1	AgeCat*Trt	Old	G1	G4	0.53	0.43	60	1.232	0.22276	Tukey-K	0.7329
T2m	1	AgeCat*Trt	Old	G1	G5	-0.4	0.43	60	-0.89	0.37802	Tukey-K	0.9002
T2m	1	AgeCat*Trt	Old	G2	G3	0.03	0.43	60	0.066	0.94723	Tukey-K	1
T2m	1	AgeCat*Trt	Old	G2	G4	0.68	0.43	60	1.591	0.11676	Tukey-K	0.5086
T2m	1	AgeCat*Trt	Old	G2	G5	-0.2	0.43	60	-0.53	0.599	Tukey-K	0.9841
T2m	1	AgeCat*Trt	Old	G3	G4	0.66	0.43	60	1.525	0.13251	Tukey-K	0.5506
T2m	1	AgeCat*Trt	Old	G3	G5	-0.3	0.43	60	-0.6	0.55401	Tukey-K	0.9753
T2m	1	AgeCat*Trt	Old	G4	G5	-0.9	0.43	60	-2.12	0.038140	Tukey-K	0.2252
T2m	1	AgeCat*Trt	Young	G1	G2	-1	0.497	60	-2.01	0.04883	Tukey-K	0.2734
T2m	1	AgeCat*Trt	Young	G1	G3	-0.4	0.497	60	-0.71	0.48019	Tukey-K	0.9533
T2m	1	AgeCat*Trt	Young	G1	G4	-0.7	0.497	60	-1.46	0.15052	Tukey-K	0.5943
T2m	1	AgeCat*Trt	Young	G1	G5	-0.7	0.497	60	-1.47	0.14672	Tukey-K	0.5855
T2m	1	AgeCat*Trt	Young	G2	G3	0.65	0.497	60	1.301	0.1984	Tukey-K	0.6919
T2m	1	AgeCat*Trt	Young	G2	G4	0.28	0.497	60	0.555	0.58122	Tukey-K	0.981
T2m	1	AgeCat*Trt	Young	G2	G5	0.27	0.497	60	0.541	0.59074	Tukey-K	0.9827
T2m	1	AgeCat*Trt	Young	G3	G4	-0.4	0.497	60	-0.75	0.45865	Tukey-K	0.9447
T2m	1	AgeCat*Trt	Young	G3	G5	-0.4	0.497	60	-0.76	0.45033	Tukey-K	0.941
T2m	1	AgeCat*Trt	Young	G4	G5	-0	0.497	60	-0.01	0.98891	Tukey-K	1
T2p	1	AgeCat*Trt	Middle	G1	G2	1.16	0.637	60	1.818	0.07407	Tukey-K	0.3731
T2p	1	AgeCat*Trt	Middle	G1	G3	1.07	0.637	60	1.679	0.09832	Tukey-K	0.4543
T2p	1	AgeCat*Trt	Middle	G1	G4	0.56	0.637	60	0.881	0.38198	Tukey-K	0.9029
T2p	1	AgeCat*Trt	Middle	G1	G5	0.97	0.637	60	1.53	0.13122	Tukey-K	0.5473
T2p	1	AgeCat*Trt	Middle	G2	G3	-0.1	0.637	60	-0.14	0.89012	Tukey-K	0.9999
T2p	1	AgeCat*Trt	Middle	G3	G4	-0.6	0.637	60	-0.94	0.35242	Tukey-K	0.8812
T2p	1	AgeCat*Trt	Middle	G2	G5	-0.2	0.637	60	-0.29	0.77458	Tukey-K	0.9985
T2p	1	AgeCat*Trt	Middle	G3	G4	-0.5	0.637	60	-0.8	0.42776	Tukey-K	0.9301
T2p	1	AgeCat*Trt	Middle	G3	G5	-0.1	0.637	60	-0.15	0.8821	Tukey-K	0.9999
T2p	1	AgeCat*Trt	Middle	G4	G5	0.41	0.637	60	0.649	0.5185	Tukey-K	0.9661
T2p	1	AgeCat*Trt	Old	G1	G2	-0.6	0.45	60	-1.28	0.20441	Tukey-K	0.7025

T2p	1	AgeCat*Trt	Old	G1	G3	-0.9	0.45	60	-2.07	0.04288	Tukey-K	0.247
T2p	1	AgeCat*Trt	Old	G1	G4	-0.1	0.45	60	-0.33	0.74299	Tukey-K	0.9974
T2p	1	AgeCat*Trt	Old	G1	G5	-0.5	0.45	60	-1.16	0.25028	Tukey-K	0.7733
T2p	1	AgeCat*Trt	Old	G2	G3	-0.4	0.45	60	-0.79	0.43508	Tukey-K	0.9338
T2p	1	AgeCat*Trt	Old	G2	G4	0.43	0.45	60	0.954	0.3441	Tukey-K	0.8745
T2p	1	AgeCat*Trt	Old	G2	G5	0.06	0.45	60	0.122	0.90319	Tukey-K	0.9999
T2p	1	AgeCat*Trt	Old	G3	G4	0.78	0.45	60	1.739	0.08709	Tukey-K	0.4182
T2p	1	AgeCat*Trt	Old	G3	G5	0.41	0.45	60	0.908	0.36754	Tukey-K	0.8928
T2p	1	AgeCat*Trt	Old	G4	G5	-0.4	0.45	60	-0.83	0.409	Tukey-K	0.9198
T2p	1	AgeCat*Trt	Young	G1	G2	0.1	0.52	60	0.197	0.84482	Tukey-K	0.9997
T2p	1	AgeCat*Trt	Young	G1	G3	0.11	0.52	60	0.213	0.8323	Tukey-K	0.9995
T2p	1	AgeCat*Trt	Young	G1	G4	-0	0.52	60	-0.06	0.95458	Tukey-K	1
T2p	1	AgeCat*Trt	Young	G1	G5	0.3	0.52	60	0.568	0.57211	Tukey-K	0.9792
T2p	1	AgeCat*Trt	Young	G2	G3	0.01	0.52	60	0.016	0.98721	Tukey-K	1
T2p	1	AgeCat*Trt	Young	G2	G4	-0.1	0.52	60	-0.25	0.80054	Tukey-K	0.9991
T2p	1	AgeCat*Trt	Young	G2	G5	0.19	0.52	60	0.371	0.71158	Tukey-K	0.9958
T2p	1	AgeCat*Trt	Young	G3	G4	-0.1	0.52	60	-0.27	0.78818	Tukey-K	0.9988
T2p	1	AgeCat*Trt	Young	G3	G5	0.18	0.52	60	0.355	0.72354	Tukey-K	0.9965
T2p	1	AgeCat*Trt	Young	G4	G5	0.33	0.52	60	0.625	0.53417	Tukey-K	0.9704
T3m	1	AgeCat*Trt	Middle	G1	G2	0.02	0.63	60	0.029	0.97705	Tukey-K	1
T3m	1	AgeCat*Trt	Middle	G1	G3	0.3	0.63	60	0.479	0.63389	Tukey-K	0.989
T3m	1	AgeCat*Trt	Middle	G1	G4	0.71	0.63	60	1.12	0.26729	Tukey-K	0.7955
T3m	1	AgeCat*Trt	Middle	G1	G5	0.3	0.63	60	0.473	0.63778	Tukey-K	0.9895
T3m	1	AgeCat*Trt	Middle	G2	G3	0.28	0.63	60	0.45	0.65446	Tukey-K	0.9913
T3m	1	AgeCat*Trt	Middle	G2	G4	0.69	0.63	60	1.091	0.2797	Tukey-K	0.8105
T3m	1	AgeCat*Trt	Middle	G2	G5	0.28	0.63	60	0.444	0.65841	Tukey-K	0.9917
T3m	1	AgeCat*Trt	Middle	G3	G4	0.4	0.63	60	0.641	0.52395	Tukey-K	0.9677
T3m	1	AgeCat*Trt	Middle	G3	G5	-0	0.63	60	-0.01	0.99563	Tukey-K	1
T3m	1	AgeCat*Trt	Middle	G4	G5	-0.4	0.63	60	-0.65	0.5204	Tukey-K	0.9666
T3m	1	AgeCat*Trt	Old	G1	G2	0.34	0.445	60	0.773	0.44239	Tukey-K	0.9373
T3m	1	AgeCat*Trt	Old	G1	G3	0.54	0.445	60	1.216	0.2288	Tukey-K	0.7422
T3m	1	AgeCat*Trt	Old	G1	G4	0.69	0.445	60	1.558	0.12455	Tukey-K	0.5299
T3m	1	AgeCat*Trt	Old	G1	G5	0.22	0.445	60	0.494	0.62302	Tukey-K	0.9876
T3m	1	AgeCat*Trt	Old	G2	G3	0.2	0.445	60	0.443	0.65966	Tukey-K	0.9918
T3m	1	AgeCat*Trt	Old	G2	G4	0.35	0.445	60	0.784	0.43585	Tukey-K	0.9341
T3m	1	AgeCat*Trt	Old	G2	G5	-0.1	0.445	60	-0.28	0.78108	Tukey-K	0.9986
T3m	1	AgeCat*Trt	Old	G3	G4	0.15	0.445	60	0.342	0.73362	Tukey-K	0.997
T3m	1	AgeCat*Trt	Old	G3	G5	-0.3	0.445	60	-0.72	0.47326	Tukey-K	0.9507
T3m	1	AgeCat*Trt	Old	G4	G5	-0.5	0.445	60	-1.06	0.29175	Tukey-K	0.8242
T3m	1	AgeCat*Trt	Young	G1	G2	-0.8	0.514	60	-1.5	0.13973	Tukey-K	0.5687
T3m	1	AgeCat*Trt	Young	G1	G3	-0.4	0.514	60	-0.86	0.39057	Tukey-K	0.9086

T3m	1	AgeCat*Trt	Young	G1	G4	-0.4	0.514	60	-0.78	0.43909	Tukey-K	0.9357
T3m	1	AgeCat*Trt	Young	G1	G5	-0.6	0.514	60	-1.25	0.21551	Tukey-K	0.7212
T3m	1	AgeCat*Trt	Young	G2	G3	0.32	0.514	60	0.632	0.52993	Tukey-K	0.9693
T3m	1	AgeCat*Trt	Young	G2	G4	0.37	0.514	60	0.718	0.47572	Tukey-K	0.9516
T3m	1	AgeCat*Trt	Young	G2	G5	0.13	0.514	60	0.245	0.80741	Tukey-K	0.9992
T3m	1	AgeCat*Trt	Young	G3	G4	0.04	0.514	60	0.086	0.93181	Tukey-K	1
T3m	1	AgeCat*Trt	Young	G3	G5	-0.2	0.514	60	-0.39	0.70018	Tukey-K	0.9951
T3m	1	AgeCat*Trt	Young	G4	G5	-0.2	0.514	60	-0.47	0.63803	Tukey-K	0.9895
T3p	1	AgeCat*Trt	Middle	G1	G2	0.45	0.571	60	0.791	0.43182	Tukey-K	0.9321
T3p	1	AgeCat*Trt	Middle	G1	G3	1.69	0.571	60	2.957	0.00444	Tukey-K	0.0346
T3p	1	AgeCat*Trt	Middle	G1	G4	0.8	0.571	60	1.403	0.16577	Tukey-K	0.6282
T3p	1	AgeCat*Trt	Middle	G1	G5	1.11	0.571	60	1.936	0.05753	Tukey-K	0.3098
T3p	1	AgeCat*Trt	Middle	G2	G3	1.24	0.571	60	2.166	0.03433	Tukey-K	0.2069
T3p	1	AgeCat*Trt	Middle	G2	G4	0.35	0.571	60	0.612	0.54312	Tukey-K	0.9727
T3p	1	AgeCat*Trt	Middle	G2	G5	0.65	0.571	60	1.145	0.25675	Tukey-K	0.782
T3p	1	AgeCat*Trt	Middle	G3	G4	-0.9	0.571	60	-1.55	0.12544	Tukey-K	0.5322
T3p	1	AgeCat*Trt	Middle	G3	G5	-0.6	0.571	60	-1.02	0.31156	Tukey-K	0.8449
T3p	1	AgeCat*Trt	Middle	G4	G5	0.3	0.571	60	0.533	0.59571	Tukey-K	0.9835
T3p	1	AgeCat*Trt	Old	G1	G2	-0	0.404	60	-0.03	0.97357	Tukey-K	1
T3p	1	AgeCat*Trt	Old	G1	G3	-0.6	0.404	60	-1.36	0.17769	Tukey-K	0.6527
T3p	1	AgeCat*Trt	Old	G1	G4	0.24	0.404	60	0.607	0.54625	Tukey-K	0.9735
T3p	1	AgeCat*Trt	Old	G1	G5	-0	0.404	60	-0.11	0.9149	Tukey-K	1
T3p	1	AgeCat*Trt	Old	G2	G3	-0.5	0.404	60	-1.33	0.18835	Tukey-K	0.6734
T3p	1	AgeCat*Trt	Old	G2	G4	0.26	0.404	60	0.64	0.52455	Tukey-K	0.9678
T3p	1	AgeCat*Trt	Old	G2	G5	-0	0.404	60	-0.07	0.94122	Tukey-K	1
T3p	1	AgeCat*Trt	Old	G3	G4	0.8	0.404	60	1.971	0.05337	Tukey-K	0.2927
T3p	1	AgeCat*Trt	t Old	G3	G5	0.51	0.404	60	1.257	0.21377	Tukey-K	0.7184
T3p	1	AgeCat*Trt	Old	G4	G5	-0.3	0.404	60	-0.71	0.47791	Tukey-K	0.9525
T3p	1	AgeCat*Trt	Young	G1	G2	-0.2	0.466	60	-0.43	0.67235	Tukey-K	0.993
T3p	1	AgeCat*Trt	Young	G1	G3	0.1	0.466	60	0.224	0.82378	Tukey-K	0.9994
T3p	1	AgeCat*Trt	Young	G1	G4	0.16	0.466	60	0.351	0.72661	Tukey-K	0.9966
T3p	1	AgeCat*Trt	Young	G1	G5	0.55	0.466	60	1.18	0.24256	Tukey-K	0.7625
T3p	1	AgeCat*Trt	Young	G2	G3	0.3	0.466	60	0.649	0.51903	Tukey-K	0.9662
T3p	1	AgeCat*Trt	Young	G2	G4	0.36	0.466	60	0.776	0.44062	Tukey-K	0.9365
T3p	1	AgeCat*Trt	Young	G2	G5	0.75	0.466	60	1.605	0.11369	Tukey-K	0.5
T3p	1	AgeCat*Trt	Young	G3	G4	0.06	0.466	60	0.128	0.89887	Tukey-K	0.9999
T3p	1	AgeCat*Trt	Young	G3	G5	0.45	0.466	60	0.957	0.34262	Tukey-K	0.8732
T3p	1	AgeCat*Trt	Young	G4	G5	0.39	0.466	60	0.829	0.41041	Tukey-K	0.9206
T4m	1	AgeCat*Trt	Middle	G1	G2	-0.4	0.545	60	-0.7	0.48671	Tukey-K	0.9557
T4m	1	AgeCat*Trt	Middle	G1	G3	-0.3	0.545	60	-0.49	0.62487	Tukey-K	0.9879
T4m	1	AgeCat*Trt	Middle	G1	G4	0.43	0.545	60	0.786	0.4351	Tukey-K	0.9338

T4m	1	AgeCat*Trt	Middle	G1	G5	-0.2	0.545	60	-0.33	0.74431	Tukey-K	0.9974
T4m	1	AgeCat*Trt	Middle	G2	G3	0.11	0.545	60	0.208	0.83565	Tukey-K	0.9996
T4m	1	AgeCat*Trt	Middle	G2	G4	0.81	0.545	60	1.486	0.14261	Tukey-K	0.5757
T4m	1	AgeCat*Trt	Middle	G2	G5	0.2	0.545	60	0.372	0.71105	Tukey-K	0.9958
T4m	1	AgeCat*Trt	Middle	G3	G4	0.7	0.545	60	1.277	0.20643	Tukey-K	0.706
T4m	1	AgeCat*Trt	Middle	G3	G5	0.09	0.545	60	0.164	0.87041	Tukey-K	0.9998
T4m	1	AgeCat*Trt	Middle	G4	G5	-0.6	0.545	60	-1.11	0.26997	Tukey-K	0.7988
T4m	1	AgeCat*Trt	Old	G1	G2	0.72	0.385	60	1.878	0.0652	Tukey-K	0.34
T4m	1	AgeCat*Trt	Old	G1	G3	0.42	0.385	60	1.085	0.2821	Tukey-K	0.8133
T4m	1	AgeCat*Trt	Old	G1	G4	0.23	0.385	60	0.593	0.55521	Tukey-K	0.9756
T4m	1	AgeCat*Trt	Old	G1	G5	0.09	0.385	60	0.245	0.80715	Tukey-K	0.9992
T4m	1	AgeCat*Trt	Old	G2	G3	-0.3	0.385	60	-0.79	0.43094	Tukey-K	0.9317
T4m	1	AgeCat*Trt	Old	G2	G4	-0.5	0.385	60	-1.29	0.20373	Tukey-K	0.7013
T4m	1	AgeCat*Trt	Old	G2	G5	-0.6	0.385	60	-1.63	0.10768	Tukey-K	0.4826
T4m	1	AgeCat*Trt	Old	G3	G4	-0.2	0.385	60	-0.49	0.62447	Tukey-K	0.9878
T4m	1	AgeCat*Trt	Old	G3	G5	-0.3	0.385	60	-0.84	0.40415	Tukey-K	0.9169
T4m	1	AgeCat*Trt	Old	G4	G5	-0.1	0.385	60	-0.35	0.72898	Tukey-K	0.9968
T4m	1	AgeCat*Trt	Young	G1	G2	0.09	0.445	60	0.194	0.84671	Tukey-K	0.9997
T4m	1	AgeCat*Trt	Young	G1	G3	0.2	0.445	60	0.449	0.65479	Tukey-K	0.9914
T4m	1	AgeCat*Trt	Young	G1	G4	-0.3	0.445	60	-0.6	0.55119	Tukey-K	0.9747
T4m	1	AgeCat*Trt	Young	G1	G5	-0.3	0.445	60	-0.6	0.54861	Tukey-K	0.9741
T4m	1	AgeCat*Trt	Young	G2	G3	0.11	0.445	60	0.255	0.79944	Tukey-K	0.999
T4m	1	AgeCat*Trt	Young	G2	G4	-0.4	0.445	60	-0.79	0.4306	Tukey-K	0.9315
T4m	1	AgeCat*Trt	Young	G2	G5	-0.4	0.445	60	-0.8	0.42835	Tukey-K	0.9304
T4m	1	AgeCat*Trt	Young	G3	G4	-0.5	0.445	60	-1.05	0.29851	Tukey-K	0.8315
T4m	1	AgeCat*Trt	Young	G3	G5	-0.5	0.445	60	-1.05	0.29674	Tukey-K	0.8296
T4m	1	AgeCat*Trt	Young	G4	G5	-0	0.445	60	-0	0.9969	Tukey-K	1
T4p	1	AgeCat*Trt	Middle	G1	G2	0.58	0.801	60	0.719	0.47511	Tukey-K	0.9514
T4p	1	AgeCat*Trt	Middle	G1	G3	0.63	0.801	60	0.783	0.43699	Tukey-K	0.9347
T4p	1	AgeCat*Trt	Middle	G1	G4	0.57	0.801	60	0.714	0.47776	Tukey-K	0.9524
T4p	1	AgeCat*Trt	Middle	G1	G5	1.21	0.801	60	1.506	0.13743	Tukey-K	0.563
T4p	1	AgeCat*Trt	Middle	G2	G3	0.05	0.801	60	0.064	0.94933	Tukey-K	1
T4p	1	AgeCat*Trt	Middle	G2	G4	-0	0.801	60	-0	0.99656	Tukey-K	1
T4p	1	AgeCat*Trt	Middle	G2	G5	0.63	0.801	60	0.787	0.43447	Tukey-K	0.9335
T4p	1	AgeCat*Trt	Middle	G3	G4	-0.1	0.801	60	-0.07	0.9459	Tukey-K	1
T4p	1	AgeCat*Trt	Middle	G3	G5	0.58	0.801	60	0.723	0.47247	Tukey-K	0.9504
T4p	1	AgeCat*Trt	Middle	G4	G5	0.63	0.801	60	0.791	0.43196	Tukey-K	0.9322
T4p	1	AgeCat*Trt	Old	G1	G2	-0.3	0.566	60	-0.44	0.6595	Tukey-K	0.9918
T4p	1	AgeCat*Trt	Old	G1	G3	-0.5	0.566	60	-0.96	0.3391	Tukey-K	0.8703
T4p	1	AgeCat*Trt	Old	G1	G4	-0.3	0.566	60	-0.53	0.6015	Tukey-K	0.9845
T4p	1	AgeCat*Trt	Old	G1	G5	-0.6	0.566	60	-1.07	0.28996	Tukey-K	0.8222

T4p	1	AgeCat*Trt	Old	G2	G3	-0.3	0.566	60	-0.52	0.60441	Tukey-K	0.9849
T4p	1	AgeCat*Trt	Old	G2	G4	-0	0.566	60	-0.08	0.93475	Tukey-K	1
T4p	1	AgeCat*Trt	Old	G2	G5	-0.4	0.566	60	-0.62	0.53445	Tukey-K	0.9705
T4p	1	AgeCat*Trt	Old	G3	G4	0.25	0.566	60	0.439	0.66252	Tukey-K	0.9921
T4p	1	AgeCat*Trt	Old	G3	G5	-0.1	0.566	60	-0.1	0.91751	Tukey-K	1
T4p	1	AgeCat*Trt	Old	G4	G5	-0.3	0.566	60	-0.54	0.5894	Tukey-K	0.9824
T4p	1	AgeCat*Trt	Young	G1	G2	-0.1	0.654	60	-0.16	0.87354	Tukey-K	0.9998
T4p	1	AgeCat*Trt	Young	G1	G3	-0.1	0.654	60	-0.11	0.90933	Tukey-K	1
T4p	1	AgeCat*Trt	Young	G1	G4	-0.2	0.654	60	-0.25	0.80521	Tukey-K	0.9991
T4p	1	AgeCat*Trt	Young	G1	G5	-0.3	0.654	60	-0.43	0.67092	Tukey-K	0.9929
T4p	1	AgeCat*Trt	Young	G2	G3	0.03	0.654	60	0.045	0.96388	Tukey-K	1
T4p	1	AgeCat*Trt	Young	G2	G4	-0.1	0.654	60	-0.09	0.93027	Tukey-K	1
T4p	1	AgeCat*Trt	Young	G2	G5	-0.2	0.654	60	-0.27	0.79028	Tukey-K	0.9988
T4p	1	AgeCat*Trt	Young	G3	G4	-0.1	0.654	60	-0.13	0.89436	Tukey-K	0.9999
T4p	1	AgeCat*Trt	Young	G3	G5	-0.2	0.654	60	-0.31	0.75566	Tukey-K	0.9979
T4p	1	AgeCat*Trt	Young	G4	G5	-0.1	0.654	60	-0.18	0.85833	Tukey-K	0.9998
T5m	1	AgeCat*Trt	Middle	G1	G2	-0.2	0.652	48	-0.27	0.79015	Tukey-K	0.9988
T5m	1	AgeCat*Trt	Middle	G1	G3	0.07	0.652	48	0.106	0.91576	Tukey-K	1
T5m	1	AgeCat*Trt	Middle	G1	G4	0.07	0.652	48	0.106	0.91576	Tukey-K	1
T5m	1	AgeCat*Trt	Middle	G1	G5	0.07	0.652	48	0.111	0.91226	Tukey-K	1
T5m	1	AgeCat*Trt	Middle	G2	G3	0.24	0.652	48	0.374	0.71009	Tukey-K	0.9957
T5m	1	AgeCat*Trt	Middle	G2	G4	0.24	0.652	48	0.374	0.71009	Tukey-K	0.9957
T5m	1	AgeCat*Trt	Middle	G2	G5	0.25	0.652	48	0.378	0.70682	Tukey-K	0.9955
T5m	1	AgeCat*Trt	Middle	G3	G4	0	0.652	48	3E-16	1	Tukey-K	1
T5m	1	AgeCat*Trt	Middle	G3	G5	0	0.652	48	0.004	0.99648	Tukey-K	1
T5m	1	AgeCat*Trt	Middle	G4	G5	0	0.652	48	0.004	0.99648	Tukey-K	1
T5m	1	AgeCat*Trt	Old	G1	G2	-0.1	0.461	48	-0.2	0.84437	Tukey-K	0.9996
T5m	1	AgeCat*Trt	Old	G1	G3	0.08	0.461	48	0.166	0.86882	Tukey-K	0.9998
T5m	1	AgeCat*Trt	Old	G1	G4	0.2	0.461	48	0.442	0.66066	Tukey-K	0.9918
T5m	1	AgeCat*Trt	Old	G1	G5	-0.1	0.461	48	-0.29	0.76965	Tukey-K	0.9983
T5m	1	AgeCat*Trt	Old	G2	G3	0.17	0.461	48	0.363	0.71789	Tukey-K	0.9961
T5m	1	AgeCat*Trt	Old	G2	G4	0.29	0.461	48	0.639	0.52579	Tukey-K	0.9678
T5m	1	AgeCat*Trt	Old	G2	G5	-0	0.461	48	-0.1	0.92304	Tukey-K	1
T5m	1	AgeCat*Trt	Old	G3	G4	0.13	0.461	48	0.276	0.78397	Tukey-K	0.9987
T5m	1	AgeCat*Trt	Old	G3	G5	-0.2	0.461	48	-0.46	0.64721	Tukey-K	0.9905
T5m	1	AgeCat*Trt	Old	G4	G5	-0.3	0.461	48	-0.74	0.46517	Tukey-K	0.9469
T5m	1	AgeCat*Trt	Young	G1	G2	0.44	0.461	48	0.963	0.34035	Tukey-K	0.8702
T5m	1	AgeCat*Trt	Young	G1	G3	0.47	0.461	48	1.019	0.3134	Tukey-K	0.8455
T5m	1	AgeCat*Trt	Young	G1	G4	-0.1	0.461	48	-0.17	0.8649	Tukey-K	0.9998
T5m	1	AgeCat*Trt	Young	G1	G5	-0.4	0.461	48	-0.95	0.3485	Tukey-K	0.877

T5m	1	AgeCat*Trt	Young	G2	G3	0.03	0.461	48	0.056	0.95576	Tukey-K	1
T5m	1	AgeCat*Trt	Young	G2	G4	-0.5	0.461	48	-1.13	0.26238	Tukey-K	0.7877
T5m	1	AgeCat*Trt	Young	G2	G5	-0.9	0.461	48	-1.91	0.06214	Tukey-K	0.3262
T5m	1	AgeCat*Trt	Young	G3	G4	-0.5	0.461	48	-1.19	0.23995	Tukey-K	0.7571
T5m	1	AgeCat*Trt	Young	G3	G5	-0.9	0.461	48	-1.97	0.05515	Tukey-K	0.2981
T5m	1	AgeCat*Trt	Young	G4	G5	-0.4	0.461	48	-0.78	0.44173	Tukey-K	0.9364
T5p	1	AgeCat*Trt	Middle	G1	G2	-0.7	0.759	48	-0.98	0.33252	Tukey-K	0.8634
T5p	1	AgeCat*Trt	Middle	G1	G3	-0.2	0.759	48	-0.26	0.7998	Tukey-K	0.999
T5p	1	AgeCat*Trt	Middle	G1	G4	-0	0.759	48	-0.06	0.9523	Tukey-K	1
T5p	1	AgeCat*Trt	Middle	G1	G5	0.59	0.759	48	0.774	0.44263	Tukey-K	0.9368
T5p	1	AgeCat*Trt	Middle	G2	G3	0.55	0.759	48	0.724	0.47263	Tukey-K	0.9499
T5p	1	AgeCat*Trt	Middle	G2	G4	0.7	0.759	48	0.919	0.3628	Tukey-K	0.8883
T5p	1	AgeCat*Trt	Middle	G2	G5	1.33	0.759	48	1.753	0.08597	Tukey-K	0.4123
T5p	1	AgeCat*Trt	Middle	G3	G4	0.15	0.759	48	0.195	0.84631	Tukey-K	0.9997
T5p	1	AgeCat*Trt	Middle	G3	G5	0.78	0.759	48	1.029	0.30856	Tukey-K	0.8406
T5p	1	AgeCat*Trt	Middle	G4	G5	0.63	0.759	48	0.834	0.40824	Tukey-K	0.9186
T5p	1	AgeCat*Trt	Old	G1	G2	-1	0.537	48	-1.95	0.05721	Tukey-K	0.3066
T5p	1	AgeCat*Trt	Old	G1	G3	-1	0.537	48	-1.81	0.07653	Tukey-K	0.3798
T5p	1	AgeCat*Trt	Old	G1	G4	-0.6	0.537	48	-1.12	0.26687	Tukey-K	0.7934
T5p	1	AgeCat*Trt	Old	G1	G5	-0.6	0.537	48	-1.05	0.30087	Tukey-K	0.8327
T5p	1	AgeCat*Trt	Old	G2	G3	0.07	0.537	48	0.138	0.89055	Tukey-K	0.9999
T5p	1	AgeCat*Trt	Old	G2	G4	0.44	0.537	48	0.825	0.41336	Tukey-K	0.9215
T5p	1	AgeCat*Trt	Old	G2	G5	0.48	0.537	48	0.903	0.37121	Tukey-K	0.8945
T5p	1	AgeCat*Trt	Old	G3	G4	0.37	0.537	48	0.687	0.4955	Tukey-K	0.9584
T5p	1	AgeCat*Trt	Old	G3	G5	0.41	0.537	48	0.764	0.44841	Tukey-K	0.9395
T5p	1	AgeCat*Trt	Old	G4	G5	0.04	0.537	48	0.078	0.93854	Tukey-K	1
T5p	1	AgeCat*Trt	Young	G1	G2	0.47	0.537	48	0.876	0.38523	Tukey-K	0.9042
T5p	1	AgeCat*Trt	Young	G1	G3	0.26	0.537	48	0.491	0.62536	Tukey-K	0.9878
T5p	1	AgeCat*Trt	Young	G1	G4	0.59	0.537	48	1.096	0.27835	Tukey-K	0.8075
T5p	1	AgeCat*Trt	Young	G1	G5	0.13	0.537	48	0.235	0.81504	Tukey-K	0.9993
T5p	1	AgeCat*Trt	Young	G2	G3	-0.2	0.537	48	-0.38	0.70204	Tukey-K	0.9952
T5p	1	AgeCat*Trt	Young	G2	G4	0.12	0.537	48	0.22	0.82669	Tukey-K	0.9995
T5p	1	AgeCat*Trt	Young	G2	G5	-0.3	0.537	48	-0.64	0.52452	Tukey-K	0.9675
T5p	1	AgeCat*Trt	Young	G3	G4	0.32	0.537	48	0.605	0.54802	Tukey-K	0.9736
T5p	1	AgeCat*Trt	Young	G3	G5	-0.1	0.537	48	-0.26	0.79888	Tukey-K	0.999
T5p	1	AgeCat*Trt	Young	G4	G5	-0.5	0.537	48	-0.86	0.3934	Tukey-K	0.9095
T6m	1	AgeCat*Trt	Middle	G1	G2	-0.7	1.11	56	-0.6	0.54853	Tukey-K	0.9739
T6m	1	AgeCat*Trt	Middle	G1	G3	-0.8	1.11	56	-0.71	0.48014	Tukey-K	0.9531
T6m	1	AgeCat*Trt	Middle	G1	G4	0.23	1.11	56	0.205	0.83818	Tukey-K	0.9996
T6m	1	AgeCat*Trt	Middle	G1	G5	-1	1.11	56	-0.88	0.38263	Tukey-K	0.903
T6m	1	AgeCat*Trt	Middle	G2	G3	-0.1	1.132	52	-0.11	0.91666	Tukey-K	1

T6m	1	AgeCat*Trt	Middle	G2	G4	0.9	1.132	52	0.793	0.43126	Tukey-K	0.9315
T6m	1	AgeCat*Trt	Middle	G2	G5	-0.3	1.132	52	-0.27	0.78744	Tukey-K	0.9988
T6m	1	AgeCat*Trt	Middle	G3	G4	1.02	1.132	52	0.898	0.37313	Tukey-K	0.8963
T6m	1	AgeCat*Trt	Middle	G3	G5	-0.2	1.132	52	-0.17	0.86889	Tukey-K	0.9998
T6m	1	AgeCat*Trt	Middle	G4	G5	-1.2	1.132	52	-1.06	0.29212	Tukey-K	0.8238
T6m	1	AgeCat*Trt	Old	G1	G2	-0.6	0.741	52	-0.84	0.40639	Tukey-K	0.9178
T6m	1	AgeCat*Trt	Old	G1	G3	-0.2	0.741	52	-0.33	0.74136	Tukey-K	0.9973
T6m	1	AgeCat*Trt	Old	G1	G4	0.23	0.741	52	0.306	0.76051	Tukey-K	0.998
T6m	1	AgeCat*Trt	Old	G1	G5	-0.7	0.741	52	-0.94	0.35123	Tukey-K	0.8797
T6m	1	AgeCat*Trt	Old	G2	G3	0.37	0.741	52	0.505	0.61553	Tukey-K	0.9865
T6m	1	AgeCat*Trt	Old	G2	G4	0.85	0.741	52	1.143	0.25806	Tukey-K	0.7828
T6m	1	AgeCat*Trt	Old	G2	G5	-0.1	0.741	52	-0.1	0.9179	Tukey-K	1
T6m	1	AgeCat*Trt	Old	G3	G4	0.47	0.741	52	0.638	0.52612	Tukey-K	0.9681
T6m	1	AgeCat*Trt	Old	G3	G5	-0.5	0.741	52	-0.61	0.54529	Tukey-K	0.9731
T6m	1	AgeCat*Trt	Old	G4	G5	-0.9	0.741	52	-1.25	0.21795	Tukey-K	0.7241
T6m	1	AgeCat*Trt	Young	G1	G2	-0.6	0.8	52	-0.74	0.46421	Tukey-K	0.9467
T6m	1	AgeCat*Trt	Young	G1	G3	0.51	0.8	52	0.637	0.52713	Tukey-K	0.9684
T6m	1	AgeCat*Trt	Young	G1	G4	-0.5	0.8	52	-0.56	0.5746	Tukey-K	0.9796
T6m	1	AgeCat*Trt	Young	G1	G5	0.56	0.8	52	0.698	0.48804	Tukey-K	0.956
T6m	1	AgeCat*Trt	Young	G2	G3	1.1	0.8	52	1.374	0.1753	Tukey-K	0.6467
T6m	1	AgeCat*Trt	Young	G2	G4	0.14	0.8	52	0.173	0.8637	Tukey-K	0.9998
T6m	1	AgeCat*Trt	Young	G2	G5	1.15	0.8	52	1.436	0.15704	Tukey-K	0.6078
T6m	1	AgeCat*Trt	Young	G3	G4	-1	0.8	52	-1.2	0.23498	Tukey-K	0.7505
T6m	1	AgeCat*Trt	Young	G3	G5	0.05	0.8	52	0.062	0.95102	Tukey-K	1
T6m	1	AgeCat*Trt	Young	G4	G5	1.01	0.8	52	1.263	0.21212	Tukey-K	0.7145
T6p	1	AgeCat*Trt	Middle	G1	G2	0.88	1.035	51	0.847	0.40073	Tukey-K	0.9143
T6p	1	AgeCat*Trt	Middle	G1	G3	1.5	1.035	51	1.452	0.15263	Tukey-K	0.5977
T6p	1	AgeCat*Trt	Middle	G1	G4	1.49	1.035	51	1.443	0.15512	Tukey-K	0.6033
T6p	1	AgeCat*Trt	Middle	G1	G5	3.09	1.035	51	2.989	0.00429	Tukey-K	0.0334
T6p	1	AgeCat*Trt	Middle	G2	G3	0.63	1.035	51	0.605	0.54815	Tukey-K	0.9737
T6p	1	AgeCat*Trt	Middle	G2	G4	0.62	1.035	51	0.596	0.55405	Tukey-K	0.9751
T6p	1	AgeCat*Trt	Middle	G2	G5	2.22	1.035	51	2.142	0.03699	Tukey-K	0.2186
T6p	1	AgeCat*Trt	Middle	G3	G4	-0	1.035	51	-0.01	0.99291	Tukey-K	1
T6p	1	AgeCat*Trt	Middle	G3	G5	1.59	1.035	51	1.537	0.13038	Tukey-K	0.5436
T6p	1	AgeCat*Trt	Middle	G4	G5	1.6	1.035	51	1.546	0.12821	Tukey-K	0.538
T6p	1	AgeCat*Trt	Old	G1	G2	-1.2	0.677	51	-1.7	0.09514	Tukey-K	0.4428
T6p	1	AgeCat*Trt	Old	G1	G3	-1.4	0.677	51	-2.1	0.04047	Tukey-K	0.2349
T6p	1	AgeCat*Trt	Old	G1	G4	-0.6	0.677	51	-0.89	0.38025	Tukey-K	0.9011
T6p	1	AgeCat*Trt	Old	G1	G5	-1.8	0.677	51	-2.66	0.01053	Tukey-K	0.0752
T6p	1	AgeCat*Trt	Old	G2	G3	-0.3	0.677	51	-0.4	0.68937	Tukey-K	0.9943
T6p	1	AgeCat*Trt	Old	G2	G4	0.55	0.677	51	0.815	0.41868	Tukey-K	0.9247

T6p	1	AgeCat*Trt	Old	G2	G5	-0.6	0.677	51	-0.96	0.34394	Tukey-K	0.8736
T6p	1	AgeCat*Trt	Old	G3	G4	0.82	0.677	51	1.217	0.22909	Tukey-K	0.7414
T6p	1	AgeCat*Trt	Old	G3	G5	-0.4	0.677	51	-0.55	0.58248	Tukey-K	0.981
T6p	1	AgeCat*Trt	Old	G4	G5	-1.2	0.677	51	-1.77	0.0826	Tukey-K	0.4016
T6p	1	AgeCat*Trt	Young	G1	G2	-0.8	0.775	51	-1	0.32296	Tukey-K	0.855
T6p	1	AgeCat*Trt	Young	G1	G3	-0.1	0.732	51	-0.15	0.87919	Tukey-K	0.9999
T6p	1	AgeCat*Trt	Young	G1	G4	-0.3	0.732	51	-0.35	0.72769	Tukey-K	0.9967
T6p	1	AgeCat*Trt	Young	G1	G5	0.02	0.732	51	0.025	0.98026	Tukey-K	1
T6p	1	AgeCat*Trt	Young	G2	G3	0.66	0.775	51	0.854	0.39715	Tukey-K	0.9121
T6p	1	AgeCat*Trt	Young	G2	G4	0.52	0.775	51	0.668	0.50735	Tukey-K	0.9624
T6p	1	AgeCat*Trt	Young	G2	G5	0.79	0.775	51	1.021	0.31183	Tukey-K	0.8443
T6p	1	AgeCat*Trt	Young	G3	G4	-0.1	0.732	51	-0.2	0.84433	Tukey-K	0.9996
T6p	1	AgeCat*Trt	Young	G3	G5	0.13	0.732	51	0.178	0.85972	Tukey-K	0.9998
T6p	1	AgeCat*Trt	Young	G4	G5	0.27	0.732	51	0.375	0.70922	Tukey-K	0.9957
T7m	1	AgeCat*Trt	Middle	G1	G2	-0.4	0.995	60	-0.41	0.68199	Tukey-K	0.9938
T7m	1	AgeCat*Trt	Middle	G1	G3	-0.3	0.906	59	-0.36	0.72259	Tukey-K	0.9964
T7m	1	AgeCat*Trt	Middle	G1	G4	0.07	0.906	59	0.074	0.94155	Tukey-K	1
T7m	1	AgeCat*Trt	Middle	G1	G5	-0.6	0.906	59	-0.72	0.47723	Tukey-K	0.9522
T7m	1	AgeCat*Trt	Middle	G2	G3	0.09	0.995	60	0.087	0.93112	Tukey-K	1
T7m	1	AgeCat*Trt	Middle	G2	G4	0.48	0.995	60	0.479	0.63381	Tukey-K	0.989
T7m	1	AgeCat*Trt	Middle	G2	G5	-0.2	0.995	60	-0.24	0.81124	Tukey-K	0.9992
T7m	1	AgeCat*Trt	Middle	G3	G4	0.39	0.906	59	0.43	0.66852	Tukey-K	0.9927
T7m	1	AgeCat*Trt	Middle	G3	G5	-0.3	0.906	59	-0.36	0.72116	Tukey-K	0.9964
T7m	1	AgeCat*Trt	Middle	G4	G5	-0.7	0.906	59	-0.79	0.4333	Tukey-K	0.9328
T7m	1	AgeCat*Trt	Old	G1	G2	0.73	0.641	59	1.146	0.25635	Tukey-K	0.7813
T7m	1	AgeCat*Trt	Old	G1	G3	2.15	0.641	59	3.354	0.0014	Tukey-K	0.0117
T7m	1	AgeCat*Trt	Old	G1	G4	1.46	0.641	59	2.278	0.02637	Tukey-K	0.1666
T7m	1	AgeCat*Trt	Old	G1	G5	0.32	0.641	59	0.493	0.62387	Tukey-K	0.9877
T7m	1	AgeCat*Trt	Old	G2	G3	1.41	0.641	59	2.208	0.03115	Tukey-K	0.1911
T7m	1	AgeCat*Trt	Old	G2	G4	0.72	0.641	59	1.132	0.26236	Tukey-K	0.7892
T7m	1	AgeCat*Trt	Old	G2	G5	-0.4	0.641	59	-0.65	0.51615	Tukey-K	0.9654
T7m	1	AgeCat*Trt	Old	G3	G4	-0.7	0.641	59	-1.08	0.28622	Tukey-K	0.818
T7m	1	AgeCat*Trt	Old	G3	G5	-1.8	0.641	59	-2.86	0.00583	Tukey-K	0.0444
T7m	1	AgeCat*Trt	Old	G4	G5	-1.1	0.641	59	-1.78	0.07942	Tukey-K	0.3919
T7m	1	AgeCat*Trt	Young	G1	G2	0.43	0.74	59	0.577	0.56638	Tukey-K	0.978
T7m	1	AgeCat*Trt	Young	G1	G3	1.04	0.74	59	1.403	0.16581	Tukey-K	0.6281
T7m	1	AgeCat*Trt	Young	G1	G4	1.32	0.74	59	1.789	0.0787	Tukey-K	0.3894
T7m	1	AgeCat*Trt	Young	G1	G5	0.11	0.74	59	0.146	0.8841	Tukey-K	0.9999
T7m	1	AgeCat*Trt	Young	G2	G3	0.61	0.74	59	0.826	0.41185	Tukey-K	0.9214
T7m	1	AgeCat*Trt	Young	G2	G4	0.9	0.74	59	1.213	0.23011	Tukey-K	0.7441
T7m	1	AgeCat*Trt	Young	G2	G5	-0.3	0.74	59	-0.43	0.66859	Tukey-K	0.9927

T7m	1	AgeCat*Trt	Young	G3	G4	0.29	0.74	59	0.386	0.7008	Tukey-K	0.9952
T7m	1	AgeCat*Trt	Young	G3	G5	-0.9	0.74	59	-1.26	0.2138	Tukey-K	0.7183
T7m	1	AgeCat*Trt	Young	G4	G5	-1.2	0.74	59	-1.64	0.10573	Tukey-K	0.4767
T7p	1	AgeCat*Trt	Middle	G1	G2	0.8	0.963	60	0.835	0.40709	Tukey-K	0.9187
T7p	1	AgeCat*Trt	Middle	G1	G3	-0.6	0.963	60	-0.65	0.51621	Tukey-K	0.9654
T7p	1	AgeCat*Trt	Middle	G1	G4	-0.1	0.963	60	-0.15	0.88184	Tukey-K	0.9999
T7p	1	AgeCat*Trt	Middle	G1	G5	0.05	0.963	60	0.048	0.96211	Tukey-K	1
T7p	1	AgeCat*Trt	Middle	G2	G3	-1.4	0.963	60	-1.49	0.142	Tukey-K	0.5742
T7p	1	AgeCat*Trt	Middle	G2	G4	-0.9	0.963	60	-0.98	0.32898	Tukey-K	0.8614
T7p	1	AgeCat*Trt	Middle	G2	G5	-0.8	0.963	60	-0.79	0.43427	Tukey-K	0.9334
T7p	1	AgeCat*Trt	Middle	G3	G4	0.48	0.963	60	0.504	0.61625	Tukey-K	0.9867
T7p	1	AgeCat*Trt	Middle	G3	G5	0.67	0.963	60	0.701	0.48615	Tukey-K	0.9555
T7p	1	AgeCat*Trt	Middle	G4	G5	0.19	0.963	60	0.197	0.8445	Tukey-K	0.9997
T7p	1	AgeCat*Trt	Old	G1	G2	-1.1	0.681	60	-1.58	0.11955	Tukey-K	0.5163
T7p	1	AgeCat*Trt	Old	G1	G3	0.13	0.681	60	0.185	0.85417	Tukey-K	0.9997
T7p	1	AgeCat*Trt	Old	G1	G4	0.55	0.681	60	0.812	0.42008	Tukey-K	0.926
T7p	1	AgeCat*Trt	Old	G1	G5	-0.3	0.681	60	-0.43	0.6713	Tukey-K	0.9929
T7p	1	AgeCat*Trt	Old	G2	G3	1.2	0.681	60	1.764	0.08286	Tukey-K	0.404
T7p	1	AgeCat*Trt	Old	G2	G4	1.63	0.681	60	2.391	0.01995	Tukey-K	0.1319
T7p	1	AgeCat*Trt	Old	G2	G5	0.78	0.681	60	1.153	0.2536	Tukey-K	0.7778
T7p	1	AgeCat*Trt	Old	G3	G4	0.43	0.681	60	0.627	0.53285	Tukey-K	0.9701
T7p	1	AgeCat*Trt	Old	G3	G5	-0.4	0.681	60	-0.61	0.54347	Tukey-K	0.9728
T7p	1	AgeCat*Trt	Old	G4	G5	-0.8	0.681	60	-1.24	0.22042	Tukey-K	0.7291
T7p	1	AgeCat*Trt	Young	G1	G2	-0.7	0.786	60	-0.9	0.37231	Tukey-K	0.8962
T7p	1	AgeCat*Trt	Young	G1	G3	0.22	0.786	60	0.278	0.78182	Tukey-K	0.9987
T7p	1	AgeCat*Trt	Young	G1	G4	0.1	0.786	60	0.125	0.90069	Tukey-K	0.9999
T7p	1	AgeCat*Trt	Young	G1	G5	-0.2	0.786	60	-0.26	0.7942	Tukey-K	0.9989
T7p	1	AgeCat*Trt	Young	G2	G3	0.93	0.786	60	1.177	0.24382	Tukey-K	0.7643
T7p	1	AgeCat*Trt	Young	G2	G4	0.8	0.786	60	1.024	0.30986	Tukey-K	0.8432
T7p	1	AgeCat*Trt	Young	G2	G5	0.5	0.786	60	0.637	0.52664	Tukey-K	0.9684
T7p	1	AgeCat*Trt	Young	G3	G4	-0.1	0.786	60	-0.15	0.87901	Tukey-K	0.9999
T7p	1	AgeCat*Trt	Young	G3	G5	-0.4	0.786	60	-0.54	0.59105	Tukey-K	0.9827
T7p	1	AgeCat*Trt	Young	G4	G5	-0.3	0.786	60	-0.39	0.69988	Tukey-K	0.9951
T8m	1	AgeCat*Trt	Middle	G1	G2	-1	1.06	60	-0.93	0.35752	Tukey-K	0.8852
T8m	1	AgeCat*Trt	Middle	G1	G3	-0.8	1.06	60	-0.77	0.44418	Tukey-K	0.9382
T8m	1	AgeCat*Trt	Middle	G1	G4	0.51	1.06	60	0.483	0.63068	Tukey-K	0.9886
T8m	1	AgeCat*Trt	Middle	G1	G5	0.15	1.06	60	0.142	0.88734	Tukey-K	0.9999
T8m	1	AgeCat*Trt	Middle	G2	G3	0.17	1.06	60	0.157	0.87578	Tukey-K	0.9999
T8m	1	AgeCat*Trt	Middle	G2	G4	1.49	1.06	60	1.41	0.16356	Tukey-K	0.6234
T8m	1	AgeCat*Trt	Middle	G2	G5	1.13	1.06	60	1.07	0.28912	Tukey-K	0.8213
T8m	1	AgeCat*Trt	Middle	G3	G4	1.33	1.06	60	1.253	0.21489	Tukey-K	0.7202

T8m	1	AgeCat*Trt	Middle	G3	G5	0.97	1.06	60	0.913	0.36515	Tukey-K	0.891
T8m	1	AgeCat*Trt	Middle	G4	G5	-0.4	1.06	60	-0.34	0.73432	Tukey-K	0.997
T8m	1	AgeCat*Trt	Old	G1	G2	0.69	0.749	60	0.919	0.36162	Tukey-K	0.8884
T8m	1	AgeCat*Trt	Old	G1	G3	0.99	0.749	60	1.321	0.19166	Tukey-K	0.6796
T8m	1	AgeCat*Trt	Old	G1	G4	-0	0.749	60	-0.01	0.99058	Tukey-K	1
T8m	1	AgeCat*Trt	Old	G1	G5	0.39	0.749	60	0.525	0.60153	Tukey-K	0.9845
T8m	1	AgeCat*Trt	Old	G2	G3	0.3	0.749	60	0.401	0.68966	Tukey-K	0.9944
T8m	1	AgeCat*Trt	Old	G2	G4	-0.7	0.749	60	-0.93	0.3555	Tukey-K	0.8837
T8m	1	AgeCat*Trt	Old	G2	G5	-0.3	0.749	60	-0.39	0.69474	Tukey-K	0.9948
T8m	1	AgeCat*Trt	Old	G3	G4	-1	0.749	60	-1.33	0.18776	Tukey-K	0.6723
T8m	1	AgeCat*Trt	Old	G3	G5	-0.6	0.749	60	-0.8	0.42941	Tukey-K	0.9309
T8m	1	AgeCat*Trt	Old	G4	G5	0.4	0.749	60	0.537	0.59336	Tukey-K	0.9831
T8m	1	AgeCat*Trt	Young	G1	G2	0.57	0.865	60	0.655	0.51522	Tukey-K	0.9651
T8m	1	AgeCat*Trt	Young	G1	G3	0.73	0.865	60	0.845	0.40135	Tukey-K	0.9153
T8m	1	AgeCat*Trt	Young	G1	G4	1.18	0.865	60	1.362	0.1782	Tukey-K	0.6537
T8m	1	AgeCat*Trt	Young	G1	G5	-0.1	0.865	60	-0.14	0.88923	Tukey-K	0.9999
T8m	1	AgeCat*Trt	Young	G2	G3	0.16	0.865	60	0.191	0.84948	Tukey-K	0.9997
T8m	1	AgeCat*Trt	Young	G2	G4	0.61	0.865	60	0.708	0.48188	Tukey-K	0.954
T8m	1	AgeCat*Trt	Young	G2	G5	-0.7	0.865	60	-0.79	0.43005	Tukey-K	0.9312
T8m	1	AgeCat*Trt	Young	G3	G4	0.45	0.865	60	0.517	0.607	Tukey-K	0.9853
T8m	1	AgeCat*Trt	Young	G3	G5	-0.9	0.865	60	-0.99	0.32854	Tukey-K	0.861
T8m	1	AgeCat*Trt	Young	G4	G5	-1.3	0.865	60	-1.5	0.1383	Tukey-K	0.5652
T8p	1	AgeCat*Trt	Middle	G1	G2	-0.2	0.915	60	-0.19	0.85295	Tukey-K	0.9997
T8p	1	AgeCat*Trt	Middle	G1	G3	-1.5	0.915	60	-1.68	0.09813	Tukey-K	0.4538
T8p	1	AgeCat*Trt	Middle	G1	G4	0.33	0.915	60	0.362	0.71859	Tukey-K	0.9962
T8p	1	AgeCat*Trt	Middle	G1	G5	0.51	0.915	60	0.555	0.58086	Tukey-K	0.9809
T8p	1	AgeCat*Trt	Middle	G2	G3	-1.4	0.915	60	-1.49	0.14042	Tukey-K	0.5704
T8p	1	AgeCat*Trt	Middle	G2	G4	0.5	0.915	60	0.548	0.58559	Tukey-K	0.9818
T8p	1	AgeCat*Trt	Middle	G2	G5	0.68	0.915	60	0.741	0.4614	Tukey-K	0.9458
T8p	1	AgeCat*Trt	Middle	G3	G4	1.87	0.915	60	2.042	0.04554	Tukey-K	0.259
T8p	1	AgeCat*Trt	Middle	G3	G5	2.04	0.915	60	2.235	0.02913	Tukey-K	0.181
T8p	1	AgeCat*Trt	Middle	G4	G5	0.18	0.915	60	0.193	0.84753	Tukey-K	0.9997
T8p	1	AgeCat*Trt	Old	G1	G2	-0.3	0.647	60	-0.5	0.61722	Tukey-K	0.9868
T8p	1	AgeCat*Trt	Old	G1	G3	-1.2	0.647	60	-1.87	0.06642	Tukey-K	0.3447
T8p	1	AgeCat*Trt	Old	G1	G4	0.08	0.647	60	0.118	0.90681	Tukey-K	1
T8p	1	AgeCat*Trt	Old	G1	G5	-0	0.647	60	-0.08	0.93885	Tukey-K	1
T8p	1	AgeCat*Trt	Old	G2	G3	-0.9	0.647	60	-1.37	0.17666	Tukey-K	0.6507
T8p	1	AgeCat*Trt	Old	G2	G4	0.4	0.647	60	0.62	0.53762	Tukey-K	0.9713
T8p	1	AgeCat*Trt	Old	G2	G5	0.28	0.647	60	0.425	0.67209	Tukey-K	0.993
T8p	1	AgeCat*Trt	Old	G3	G4	1.29	0.647	60	1.987	0.05147	Tukey-K	0.2847

T8p	1	AgeCat*Trt	Old	G3	G5	1.16	0.647	60	1.793	0.07808	Tukey-K	0.3874
T8p	1	AgeCat*Trt	Old	G4	G5	-0.1	0.647	60	-0.19	0.84636	Tukey-K	0.9997
T8p	1	AgeCat*Trt	Young	G1	G2	0.66	0.747	60	0.882	0.3812	Tukey-K	0.9024
T8p	1	AgeCat*Trt	Young	G1	G3	0.33	0.747	60	0.439	0.66226	Tukey-K	0.9921
T8p	1	AgeCat*Trt	Young	G1	G4	0.22	0.747	60	0.301	0.76483	Tukey-K	0.9982
T8p	1	AgeCat*Trt	Young	G1	G5	0.19	0.747	60	0.25	0.80326	Tukey-K	0.9991
T8p	1	AgeCat*Trt	Young	G2	G3	-0.3	0.747	60	-0.44	0.6592	Tukey-K	0.9918
T8p	1	AgeCat*Trt	Young	G2	G4	-0.4	0.747	60	-0.58	0.56296	Tukey-K	0.9773
T8p	1	AgeCat*Trt	Young	G2	G5	-0.5	0.747	60	-0.63	0.52982	Tukey-K	0.9693
T8p	1	AgeCat*Trt	Young	G3	G4	-0.1	0.747	60	-0.14	0.89034	Tukey-K	0.9999
T8p	1	AgeCat*Trt	Young	G3	G5	-0.1	0.747	60	-0.19	0.85094	Tukey-K	0.9997
T8p	1	AgeCat*Trt	Young	G4	G5	-0	0.747	60	-0.05	0.96007	Tukey-K	1
T9m	1	AgeCat*Trt	Middle	G1	G2	-0.4	0.693	60	-0.54	0.59379	Tukey-K	0.9832
T9m	1	AgeCat*Trt	Middle	G1	G3	-0.4	0.693	60	-0.56	0.58014	Tukey-K	0.9807
T9m	1	AgeCat*Trt	Middle	G1	G4	1.04	0.693	60	1.505	0.1376	Tukey-K	0.5634
T9m	1	AgeCat*Trt	Middle	G1	G5	-0.8	0.693	60	-1.22	0.22774	Tukey-K	0.7406
T9m	1	AgeCat*Trt	Middle	G2	G3	-0	0.693	60	-0.02	0.98411	Tukey-K	1
T9m	1	AgeCat*Trt	Middle	G2	G4	1.41	0.693	60	2.041	0.04565	Tukey-K	0.2595
T9m	1	AgeCat*Trt	Middle	G2	G5	-0.5	0.693	60	-0.68	0.49758	Tukey-K	0.9595
T9m	1	AgeCat*Trt	Middle	G3	G4	1.43	0.693	60	2.061	0.04363	Tukey-K	0.2505
T9m	1	AgeCat*Trt	Middle	G3	G5	-0.5	0.693	60	-0.66	0.51022	Tukey-K	0.9636
T9m	1	AgeCat*Trt	Middle	G4	G5	-1.9	0.693	60	-2.72	0.00845	Tukey-K	0.0622
T9m	1	AgeCat*Trt	Old	G1	G2	0.23	0.49	60	0.466	0.64306	Tukey-K	0.9901
T9m	1	AgeCat*Trt	Old	G1	G3	0.34	0.49	60	0.701	0.48609	Tukey-K	0.9555
T9m	1	AgeCat*Trt	Old	G1	G4	0.6	0.49	60	1.226	0.22488	Tukey-K	0.7362
T9m	1	AgeCat*Trt	Old	G1	G5	0.3	0.49	60	0.615	0.54079	Tukey-K	0.9721
T9m	1	AgeCat*Trt	Old	G2	G3	0.12	0.49	60	0.235	0.81493	Tukey-K	0.9993
T9m	1	AgeCat*Trt	Old	G2	G4	0.37	0.49	60	0.761	0.44992	Tukey-K	0.9408
T9m	1	AgeCat*Trt	Old	G2	G5	0.07	0.49	60	0.149	0.88177	Tukey-K	0.9999
T9m	1	AgeCat*Trt	Old	G3	G4	0.26	0.49	60	0.525	0.60122	Tukey-K	0.9844
T9m	1	AgeCat*Trt	Old	G3	G5	-0	0.49	60	-0.09	0.93197	Tukey-K	1
T9m	1	AgeCat*Trt	Old	G4	G5	-0.3	0.49	60	-0.61	0.5434	Tukey-K	0.9728
T9m	1	AgeCat*Trt	Young	G1	G2	0.35	0.566	60	0.62	0.53762	Tukey-K	0.9713
T9m	1	AgeCat*Trt	Young	G1	G3	0.6	0.566	60	1.069	0.28957	Tukey-K	0.8218
T9m	1	AgeCat*Trt	Young	G1	G4	-0.3	0.566	60	-0.51	0.60925	Tukey-K	0.9857
T9m	1	AgeCat*Trt	Young	G1	G5	-0.2	0.566	60	-0.31	0.75552	Tukey-K	0.9979
T9m	1	AgeCat*Trt	Young	G2	G3	0.25	0.566	60	0.449	0.65539	Tukey-K	0.9914
T9m	1	AgeCat*Trt	Young	G2	G4	-0.6	0.566	60	-1.13	0.26138	Tukey-K	0.788
T9m	1	AgeCat*Trt	Young	G2	G5	-0.5	0.566	60	-0.93	0.35467	Tukey-K	0.883
T9m	1	AgeCat*Trt	Young	G3	G4	-0.9	0.566	60	-1.58	0.11882	Tukey-K	0.5143
T9m	1	AgeCat*Trt	Young	G3	G5	-0.8	0.566	60	-1.38	0.17231	Tukey-K	0.6418

T9m	1	AgeCat*Trt	Young	G4	G5	0.11	0.566	60	0.201	0.84134	Tukey-K	0.9996
T9p	1	AgeCat*Trt	Middle	G1	G2	-0.4	0.951	60	-0.39	0.69892	Tukey-K	0.995
T9p	1	AgeCat*Trt	Middle	G1	G3	-0.9	0.951	60	-0.91	0.36893	Tukey-K	0.8938
T9p	1	AgeCat*Trt	Middle	G1	G4	-0.2	0.951	60	-0.16	0.87467	Tukey-K	0.9999
T9p	1	AgeCat*Trt	Middle	G1	G5	0.33	0.951	60	0.347	0.72968	Tukey-K	0.9968
T9p	1	AgeCat*Trt	Middle	G2	G3	-0.5	0.951	60	-0.52	0.6073	Tukey-K	0.9854
T9p	1	AgeCat*Trt	Middle	G2	G4	0.22	0.951	60	0.23	0.81869	Tukey-K	0.9994
T9p	1	AgeCat*Trt	Middle	G2	G5	0.7	0.951	60	0.736	0.46472	Tukey-K	0.9472
T9p	1	AgeCat*Trt	Middle	G3	G4	0.71	0.951	60	0.747	0.45805	Tukey-K	0.9444
T9p	1	AgeCat*Trt	Middle	G3	G5	1.19	0.951	60	1.252	0.21526	Tukey-K	0.7208
T9p	1	AgeCat*Trt	Middle	G4	G5	0.48	0.951	60	0.506	0.61501	Tukey-K	0.9865
T9p	1	AgeCat*Trt	Old	G1	G2	-0.8	0.672	60	-1.19	0.23779	Tukey-K	0.7556
T9p	1	AgeCat*Trt	Old	G1	G3	-0.3	0.672	60	-0.43	0.66891	Tukey-K	0.9927
T9p	1	AgeCat*Trt	Old	G1	G4	0.33	0.672	60	0.484	0.63046	Tukey-K	0.9886
T9p	1	AgeCat*Trt	Old	G1	G5	0.51	0.672	60	0.76	0.45005	Tukey-K	0.9409
T9p	1	AgeCat*Trt	Old	G2	G3	0.51	0.672	60	0.763	0.44865	Tukey-K	0.9402
T9p	1	AgeCat*Trt	Old	G2	G4	1.13	0.672	60	1.676	0.09894	Tukey-K	0.4563
T9p	1	AgeCat*Trt	Old	G2	G5	1.31	0.672	60	1.953	0.05552	Tukey-K	0.3016
T9p	1	AgeCat*Trt	Old	G3	G4	0.61	0.672	60	0.913	0.36473	Tukey-K	0.8907
T9p	1	AgeCat*Trt	Old	G3	G5	0.8	0.672	60	1.19	0.23871	Tukey-K	0.757
T9p	1	AgeCat*Trt	Old	G4	G5	0.19	0.672	60	0.277	0.78293	Tukey-K	0.9987
T9p	1	AgeCat*Trt	Young	G1	G2	-0	0.776	60	-0.06	0.95126	Tukey-K	1
T9p	1	AgeCat*Trt	Young	G1	G3	-0.3	0.776	60	-0.35	0.73086	Tukey-K	0.9969
T9p	1	AgeCat*Trt	Young	G1	G4	0.41	0.776	60	0.523	0.60314	Tukey-K	0.9847
T9p	1	AgeCat*Trt	Young	G1	G5	-0.7	0.776	60	-0.92	0.36282	Tukey-K	0.8893
T9p	1	AgeCat*Trt	Young	G2	G3	-0.2	0.776	60	-0.28	0.77723	Tukey-K	0.9985
T9p	1	AgeCat*Trt	Young	G2	G4	0.45	0.776	60	0.584	0.56138	Tukey-K	0.977
T9p	1	AgeCat*Trt	Young	G2	G5	-0.7	0.776	60	-0.86	0.39562	Tukey-K	0.9117
T9p	1	AgeCat*Trt	Young	G3	G4	0.67	0.776	60	0.868	0.38872	Tukey-K	0.9074
T9p	1	AgeCat*Trt	Young	G3	G5	-0.4	0.776	60	-0.57	0.56987	Tukey-K	0.9787
T9p	1	AgeCat*Trt	Young	G4	G5	-1.1	0.776	60	-1.44	0.15517	Tukey-K	0.6049

SUM STATS:

GAVE THE VALUES THAT WE NEED TO PLOTTED WITH STANDARD ERROR

- Back transformed means can be substantially lower than the means in the original scale, particularly when transformation is log

Transformation: adding a constant is required to take logs when there are zero's in the data

SUM STATS

Var	AgeCat	Trt	n	mean_Y	sd_Y	SE_Y	mean_logY	sd_logY	SE_logY
T10m	Middle	G1	4	0.6086	0.2757	0.1379	-0.59	0.5353	0.2677
T10m	Middle	G2	4	0.6959	0.5016	0.2508	-0.57	0.7489	0.3745
T10m	Middle	G3	4	0.7848	0.8599	0.4299	-0.72	1.1725	0.5862
T10m	Middle	G4	4	0.4226	0.4414	0.2207	-1.21	0.9221	0.4611
T10m	Middle	G5	4	0.9513	0.7054	0.3527	-0.34	0.9799	0.4899
T10m	Old	G1	8	3.4713	4.8926	1.7298	0.631	1.1473	0.4056
T10m	Old	G2	8	1.9068	2.9553	1.0449	-0.7	2.7561	0.9744
T10m	Old	G3	8	2.8869	6.2794	2.2201	-0.97	3.0594	1.0817
T10m	Old	G4	8	4.0258	9.1323	3.2288	-0.78	2.9343	1.0374
T10m	Old	G5	8	4.2961	10.092	3.5681	-0.68	2.8982	1.0247
T10m	Young	G1	6	3.2545	3.8733	1.5813	0.413	1.5365	0.6273
T10m	Young	G2	6	1.836	1.8812	0.768	0.002	1.387	0.5662
T10m	Young	G3	6	1.2564	1.3201	0.5389	-0.38	1.3688	0.5588
T10m	Young	G4	6	1.5273	1.507	0.6152	-0.07	1.1443	0.4672
T10m	Young	G5	6	2.3776	2.8053	1.1452	0.112	1.4293	0.5835
T10p	Middle	G1	4	1.9015	1.9917	0.9959	0.267	0.9755	0.4877
T10p	Middle	G2	4	5.1492	8.2997	4.1499	0.583	1.6502	0.8251
T10p	Middle	G3	4	1.1028	0.6761	0.3381	-0.02	0.5436	0.2718
T10p	Middle	G4	4	2.1512	1.5657	0.7829	0.548	0.784	0.392
T10p	Middle	G5	4	1.8189	1.7703	0.8851	-0.12	1.6226	0.8113
T10p	Old	G1	7	1.8769	1.7452	0.6596	0.154	1.2004	0.4537
T10p	Old	G2	8	12.985	29.331	10.37	0.695	1.9537	0.6907
T10p	Old	G3	8	12.468	30.472	10.774	0.476	1.9675	0.6956
T10p	Old	G4	8	1.3931	1.5098	0.5338	-0.18	1.082	0.3826
T10p	Old	G5	8	12.761	22.265	7.872	0.539	2.2399	0.7919
T10p	Young	G1	6	2.6641	4.4624	1.8218	-0.28	1.9959	0.8148
T10p	Young	G2	6	0.7213	0.3955	0.1615	-0.55	0.874	0.3568
T10p	Young	G3	6	0.5543	0.329	0.1343	-0.94	1.1861	0.4842
T10p	Young	G4	6	0.5518	0.2681	0.1095	-0.93	1.243	0.5074
T10p	Young	G5	6	0.5691	0.5202	0.2124	-1.15	1.3431	0.5483
T1m	Middle	G1	4	1.4394	1.3081	0.6541	0.0026	1.02	0.51
T1m	Middle	G2	4	1.6605	1.7848	0.8924	0.136	0.9413	0.4707
T1m	Middle	G3	4	1.6846	1.463	0.7315	-0.17	1.7698	0.8849
T1m	Middle	G4	4	1.435	1.9386	0.9693	-0.41	1.4761	0.738
T1m	Middle	G5	4	1.7384	1.0182	0.5091	0.346	0.8395	0.4198
T1m	Old	G1	8	1.556	2.0663	0.7305	-0.199	1.2514	0.4424
T1m	Old	G2	8	0.8906	0.6482	0.2292	-0.35	0.746	0.2637
T1m	Old	G3	8	0.8256	0.6114	0.2161	-0.5	0.9015	0.3187
T1m	Old	G4	8	0.6369	0.5313	0.1878	-0.91	1.1609	0.4104
T1m	Old	G5	8	1.7706	2.5111	0.8878	-0.13	1.2509	0.4423

T1m	Young	G1	6	1.95	2.913	1.1892	0.032	1.0944	0.4468
T1m	Young	G2	6	1.5712	1.1161	0.4556	0.28	0.6149	0.251
T1m	Young	G3	6	0.9101	0.3996	0.1631	-0.21	0.5669	0.2314
T1m	Young	G4	6	2.6444	2.6136	1.067	0.581	0.9826	0.4012
T1m	Young	G5	6	1.8646	1.5972	0.6521	0.152	1.3066	0.5334
T1p	Middle	G1	4	2.4276	2.3606	1.1803	0.424	1.184	0.592
T1p	Middle	G2	4	0.9628	0.7186	0.3593	-0.29	0.8809	0.4405
T1p	Middle	G3	4	0.7572	0.585	0.2925	-0.63	1.0763	0.5382
T1p	Middle	G4	4	0.967	0.6588	0.3294	-0.24	0.7724	0.3862
T1p	Middle	G5	4	1.0003	1.117	0.5585	-0.59	1.3408	0.6704
T1p	Old	G1	8	3.4957	7.2361	2.5584	-0.05	1.5816	0.5592
T1p	Old	G2	8	2.784	5.8565	2.0706	-0.34	1.6748	0.5921
T1p	Old	G3	8	1.6201	2.3484	0.8303	-0.05	0.9653	0.3413
T1p	Old	G4	8	0.921	0.8663	0.3063	-0.7	1.4705	0.5199
T1p	Old	G5	8	1.3765	1.3353	0.4721	-0.23	1.3619	0.4815
T1p	Young	G1	5	1.7273	1.4999	0.6708	0.177	0.9982	0.4464
T1p	Young	G2	6	4.4525	7.9066	3.2279	0.378	1.5109	0.6168
T1p	Young	G3	6	6.5568	13.707	5.5959	0.55	1.4719	0.6009
T1p	Young	G4	6	15.42	34.492	14.081	0.697	1.9951	0.8145
T1p	Young	G5	6	18.184	42.65	17.412	0.415	2.153	0.879
T2m	Middle	G1	4	2.2436	2.3226	1.1613	0.235	1.367	0.6835
T2m	Middle	G2	4	2.0207	1.5519	0.7759	0.51	0.693	0.3465
T2m	Middle	G3	4	0.8261	0.6356	0.3178	-0.56	1.1707	0.5854
T2m	Middle	G4	4	0.9173	0.6789	0.3394	-0.37	0.9277	0.4638
T2m	Middle	G5	4	1.3583	0.9094	0.4547	0.122	0.7209	0.3605
T2m	Old	G1	8	1.0978	1.0548	0.3729	-0.47	1.3461	0.4759
T2m	Old	G2	8	0.9113	0.684	0.2418	-0.32	0.7286	0.2576
T2m	Old	G3	8	0.9313	0.7725	0.2731	-0.35	0.7868	0.2782
T2m	Old	G4	8	0.4148	0.2117	0.0748	-1	0.561	0.1984
T2m	Old	G5	8	3.5746	8.2617	2.921	-0.09	1.4141	0.5
T2m	Young	G1	6	2.9873	5.3324	2.1769	-0.05	1.7342	0.708
T2m	Young	G2	6	3.1379	2.1546	0.8796	0.949	0.6828	0.2787
T2m	Young	G3	6	1.7748	1.2556	0.5126	0.303	0.917	0.3744
T2m	Young	G4	6	2.712	2.2205	0.9065	0.674	0.9384	0.3831
T2m	Young	G5	6	2.7179	1.9331	0.7892	0.68	1.029	0.4201
T2p	Middle	G1	4	1.7821	1.5526	0.7763	0.149	1.1533	0.5766
T2p	Middle	G2	4	0.6143	0.5085	0.2542	-1.01	1.4706	0.7353
T2p	Middle	G3	4	0.8881	0.9809	0.4904	-0.92	1.8389	0.9194
T2p	Middle	G4	4	0.8844	0.7857	0.3928	-0.41	0.867	0.4335
T2p	Middle	G5	4	0.7825	0.9049	0.4525	-0.83	1.3416	0.6708
T2p	Old	G1	8	5.4255	13.008	4.5991	-0.44	2.1423	0.7574
T2p	Old	G2	8	3.342	6.8895	2.4358	0.142	1.3373	0.4728
T2p	Old	G3	8	4.0305	7.3766	2.608	0.496	1.2928	0.4571

T2p	Old	G4	8	1.494	2.1132	0.7471	-0.29	1.2524	0.4428
T2p	Old	G5	8	2.3041	2.3095	0.8165	0.087	1.613	0.5703
T2p	Young	G1	6	9.4224	14.244	5.8151	0.849	1.9146	0.7816
T2p	Young	G2	6	9.0677	15.899	6.4909	0.747	1.8616	0.76
T2p	Young	G3	6	3.926	4.5053	1.8393	0.738	1.2301	0.5022
T2p	Young	G4	6	7.1101	9.8837	4.035	0.879	1.6611	0.6781
T2p	Young	G5	6	5.6778	7.9088	3.2288	0.553	1.7279	0.7054
T3m	Middle	G1	4	2.6864	2.3218	1.1609	0.578	1.1194	0.5597
T3m	Middle	G2	4	2.5317	2.6569	1.3285	0.56	0.9611	0.4806
T3m	Middle	G3	4	3.2546	3.1048	1.5524	0.276	2.1465	1.0732
T3m	Middle	G4	4	1.4107	1.5771	0.7886	-0.13	1.1108	0.5554
T3m	Middle	G5	4	2.1661	2.5001	1.2501	0.28	1.1521	0.576
T3m	Old	G1	8	2.2298	2.8312	1.001	0.086	1.4593	0.5159
T3m	Old	G2	8	1.1474	0.9394	0.3321	-0.26	1.0455	0.3696
T3m	Old	G3	8	0.9162	0.6214	0.2197	-0.46	1.0691	0.378
T3m	Old	G4	8	0.8304	0.6578	0.2326	-0.61	1.1283	0.3989
T3m	Old	G5	8	2.9351	5.0638	1.7903	-0.13	1.7127	0.6055
T3m	Young	G1	6	5.3772	9.9352	4.056	0.116	2.0977	0.8564
T3m	Young	G2	6	4.053	4.9756	2.0313	0.885	1.0392	0.4242
T3m	Young	G3	6	2.5174	1.8254	0.7452	0.561	1.0981	0.4483
T3m	Young	G4	6	2.7737	1.7832	0.728	0.516	1.5036	0.6138
T3m	Young	G5	6	3.3229	3.0062	1.2273	0.76	1.1616	0.4742
T3p	Middle	G1	4	2.5481	1.9064	0.9532	0.688	0.8428	0.4214
T3p	Middle	G2	4	1.7141	1.5873	0.7936	0.236	0.8901	0.445
T3p	Middle	G3	4	0.7838	0.9234	0.4617	-1	1.688	0.844
T3p	Middle	G4	4	1.8043	2.4085	1.2043	-0.11	1.3991	0.6995
T3p	Middle	G5	4	1.2521	1.4068	0.7034	-0.42	1.4169	0.7085
T3p	Old	G1	8	5.1572	11.351	4.0132	-0.08	1.8278	0.6462
T3p	Old	G2	8	2.9249	6.0713	2.1465	-0.06	1.4193	0.5018
T3p	Old	G3	8	3.8544	6.696	2.3674	0.474	1.2842	0.454
T3p	Old	G4	8	1.8278	3.2053	1.1333	-0.32	1.3872	0.4905
T3p	Old	G5	8	1.9506	2.4779	0.8761	-0.03	1.3974	0.494
T3p	Young	G1	6	7.649	16.273	6.6432	0.569	1.5819	0.6458
T3p	Young	G2	6	6.8758	12.049	4.9189	0.767	1.5702	0.641
T3p	Young	G3	6	5.4369	10.99	4.4865	0.464	1.4415	0.5885
T3p	Young	G4	6	5.1402	10.085	4.1173	0.405	1.5202	0.6206
T3p	Young	G5	6	8.6649	19.81	8.0874	0.018	2.0075	0.8196
T4m	Middle	G1	4	0.9391	0.5648	0.2824	-0.21	0.6339	0.317
T4m	Middle	G2	4	1.2734	0.5155	0.2577	0.173	0.4375	0.2187
T4m	Middle	G3	4	1.3225	0.9107	0.4554	0.06	0.794	0.397
T4m	Middle	G4	4	0.615	0.3269	0.1635	-0.64	0.6897	0.3449
T4m	Middle	G5	4	1.0611	0.4682	0.2341	-0.03	0.5136	0.2568
T4m	Old	G1	8	2.7906	4.0831	1.4436	0.209	1.3853	0.4898

T4m	Old	G2	8	1.5349	2.5776	0.9113	-0.51	1.4939	0.5282
T4m	Old	G3	8	1.1804	1.0605	0.3749	-0.21	0.9874	0.3491
T4m	Old	G4	8	2.4215	3.6892	1.3043	-0.02	1.3657	0.4829
T4m	Old	G5	8	2.5391	4.219	1.4916	0.115	1.2917	0.4567
T4m	Young	G1	6	1.5825	1.6153	0.6594	0.105	0.8898	0.3632
T4m	Young	G2	6	1.3598	1.3084	0.5342	0.019	0.7835	0.3199
T4m	Young	G3	6	1.1994	0.9578	0.391	-0.09	0.8292	0.3385
T4m	Young	G4	6	1.6322	0.7807	0.3187	0.372	0.5619	0.2294
T4m	Young	G5	6	1.9721	1.7956	0.733	0.374	0.8597	0.351
T4p	Middle	G1	4	1.4034	0.6328	0.3164	0.259	0.4676	0.2338
T4p	Middle	G2	4	0.9256	0.6238	0.3119	-0.32	0.8813	0.4406
T4p	Middle	G3	4	0.8488	0.5227	0.2614	-0.37	0.8202	0.4101
T4p	Middle	G4	4	0.8546	0.5444	0.2722	-0.31	0.65	0.325
T4p	Middle	G5	4	0.4694	0.2916	0.1458	-0.95	0.7677	0.3839
T4p	Old	G1	8	3.5892	5.5263	1.9538	-0.54	2.9954	1.059
T4p	Old	G2	8	1.2462	1.5193	0.5371	-0.29	1.0989	0.3885
T4p	Old	G3	8	1.3579	1.2579	0.4448	0.004	0.7892	0.279
T4p	Old	G4	8	0.9759	0.5564	0.1967	-0.24	0.8307	0.2937
T4p	Old	G5	8	2.1293	2.8488	1.0072	0.063	1.3188	0.4663
T4p	Young	G1	6	9.1608	20.083	8.1989	0.413	1.8445	0.753
T4p	Young	G2	6	6.2849	12.249	5.0007	0.518	1.6303	0.6656
T4p	Young	G3	6	6.1322	12.572	5.1326	0.488	1.531	0.625
T4p	Young	G4	6	7.01	13.633	5.5656	0.575	1.7242	0.7039
T4p	Young	G5	6	11.555	23.372	9.5417	0.693	2.0363	0.8313
T5m	Middle	G1	3	4.1685	5.8714	3.3899	0.626	1.5364	0.887
T5m	Middle	G2	3	3.7708	3.9714	2.2929	0.8	1.3876	0.8011
T5m	Middle	G3	3	3.5822	3.7801	2.1824	0.556	1.7716	1.0228
T5m	Middle	G4	3	3.0265	3.6796	2.1244	0.556	1.2854	0.7421
T5m	Middle	G5	3	3.1872	3.4184	1.9736	0.553	1.5343	0.8858
T5m	Old	G1	6	4.2134	5.7112	2.3316	0.582	1.4951	0.6104
T5m	Old	G2	6	6.0108	8.8038	3.5941	0.673	1.7627	0.7196
T5m	Old	G3	6	2.6293	2.5192	1.0285	0.505	1.1308	0.4616
T5m	Old	G4	6	3.5388	3.4782	1.42	0.378	1.8189	0.7426
T5m	Old	G5	6	10.789	15.975	6.5219	0.718	2.4405	0.9963
T5m	Young	G1	6	1.8931	1.5676	0.64	-0.21	2.1507	0.878
T5m	Young	G2	6	1.3249	1.0099	0.4123	-0.66	2.4096	0.9837
T5m	Young	G3	6	1.4188	1.4228	0.5808	-0.68	2.3211	0.9476
T5m	Young	G4	6	1.9508	1.5157	0.6188	-0.13	2.0914	0.8538
T5m	Young	G5	6	2.6708	1.6021	0.654	0.225	2.1808	0.8903
T5p	Middle	G1	3	0.9315	0.5336	0.3081	-0.22	0.7258	0.4191
T5p	Middle	G2	3	2.0844	1.6407	0.9472	0.521	0.8034	0.4638
T5p	Middle	G3	3	1.96	2.6293	1.518	-0.03	1.4407	0.8318
T5p	Middle	G4	3	0.9771	0.5384	0.3108	-0.18	0.7434	0.4292

T5p	Middle	G5	3	0.5004	0.3118	0.18	-0.81	0.5718	0.3301
T5p	Old	G1	6	3.1066	3.8926	1.5891	0.126	1.9163	0.7823
T5p	Old	G2	6	12.698	24.211	9.8841	1.172	1.6823	0.6868
T5p	Old	G3	6	22.521	43.758	17.864	1.098	2.4033	0.9811
T5p	Old	G4	6	2.4962	1.7336	0.7077	0.729	0.6484	0.2647
T5p	Old	G5	6	17.253	38.92	15.889	0.688	2.1411	0.8741
T5p	Young	G1	6	1.411	1.0248	0.4184	-0.31	1.812	0.7398
T5p	Young	G2	6	0.8666	0.5751	0.2348	-0.78	1.847	0.754
T5p	Young	G3	6	1.3326	1.2393	0.5059	-0.57	2.1004	0.8575
T5p	Young	G4	6	0.9161	0.7085	0.2893	-0.89	2.1688	0.8854
T5p	Young	G5	6	1.2841	1.3206	0.5391	-0.43	1.4871	0.6071
T6m	Middle	G1	4	1.2275	1.3888	0.6944	-0.29	1.1711	0.5855
T6m	Middle	G2	3	1.9326	0.3738	0.2158	0.645	0.2067	0.1193
T6m	Middle	G3	3	3.4844	2.6835	1.5493	0.764	1.4741	0.8511
T6m	Middle	G4	3	1.012	0.7204	0.4159	-0.25	0.9924	0.573
T6m	Middle	G5	3	2.8971	1.7514	1.0112	0.952	0.5623	0.3247
T6m	Old	G1	7	1.6815	1.8773	0.7096	0.007	1.1173	0.4223
T6m	Old	G2	7	13.636	30.156	11.398	0.628	2.3132	0.8743
T6m	Old	G3	7	11.275	26.145	9.8819	0.253	2.4702	0.9337
T6m	Old	G4	7	25.749	65.095	24.603	-0.22	3.2973	1.2463
T6m	Old	G5	7	233.84	589.98	222.99	0.704	4.4493	1.6817
T6m	Young	G1	6	4.6975	4.3684	1.7834	1.028	1.2639	0.516
T6m	Young	G2	6	6.5898	5.807	2.3707	1.618	0.7735	0.3158
T6m	Young	G3	6	4.4263	4.8791	1.9919	0.518	1.8793	0.7672
T6m	Young	G4	6	14.307	26.714	10.906	1.48	1.5778	0.6441
T6m	Young	G5	6	16.345	34.431	14.056	0.469	2.5101	1.0248
T6p	Middle	G1	3	6.7043	7.9098	4.5667	1.424	1.1627	0.6713
T6p	Middle	G2	3	1.9609	1.1478	0.6627	0.548	0.6299	0.3637
T6p	Middle	G3	3	2.4548	3.5291	2.0375	-0.08	1.8078	1.0438
T6p	Middle	G4	3	2.0087	1.6855	0.9731	-0.07	1.996	1.1524
T6p	Middle	G5	3	1.5183	2.5623	1.4793	-1.67	2.7458	1.5853
T6p	Old	G1	7	2.5617	2.8083	1.0614	0.037	1.7579	0.6644
T6p	Old	G2	7	16.392	32.141	12.148	1.189	1.9749	0.7464
T6p	Old	G3	7	54.479	98.114	37.084	1.462	2.8642	1.0825
T6p	Old	G4	7	60.48	150.26	56.793	0.637	3.0872	1.1668
T6p	Old	G5	7	216.95	526.67	199.06	1.836	3.1395	1.1866
T6p	Young	G1	6	5.0019	10.711	4.3726	0.116	1.5982	0.6525
T6p	Young	G2	5	6.498	6.7021	2.9973	1.057	1.6876	0.7547
T6p	Young	G3	6	5.2806	10.099	4.1228	0.228	1.8091	0.7385
T6p	Young	G4	6	4.9603	8.3847	3.423	0.373	1.6715	0.6824
T6p	Young	G5	6	4.1164	7.6693	3.131	0.098	1.8075	0.7379
T7m	Middle	G1	4	1.3708	0.9449	0.4725	0.168	0.5933	0.2966
T7m	Middle	G2	4	3.3834	4.8413	2.4206	0.828	1.6011	0.9244

T7m	Middle	G3	4	3.8196	3.9737	1.9868	0.491	1.7337	0.8668
T7m	Middle	G4	4	2.0127	2.0397	1.0199	0.101	1.3986	0.6993
T7m	Middle	G5	4	3.4043	2.9301	1.4651	0.816	1.1397	0.5698
T7m	Old	G1	8	4.5137	7.0197	2.4819	0.595	1.534	0.5423
T7m	Old	G2	8	2.4673	2.4891	0.88	-0.14	2.2123	0.7822
T7m	Old	G3	8	1.7041	2.6606	0.9407	-1.55	3.4585	1.2228
T7m	Old	G4	8	1.9803	2.5301	0.8945	-0.86	2.6848	0.9492
T7m	Old	G5	8	23.275	59.591	21.068	0.279	2.9728	1.0511
T7m	Young	G1	6	10.428	15.871	6.4791	1.398	1.7444	0.7122
T7m	Young	G2	6	6.3734	6.8565	2.7991	0.971	1.764	0.7201
T7m	Young	G3	6	2.8707	2.9613	1.209	0.36	1.5191	0.6202
T7m	Young	G4	6	1.7423	1.523	0.6218	0.074	1.3256	0.5412
T7m	Young	G5	6	12.922	20.872	8.5209	1.289	2.2152	0.9043
T7p	Middle	G1	4	2.298	2.2132	1.1066	0.463	0.9989	0.4995
T7p	Middle	G2	4	1.3873	1.6726	0.8363	-0.34	1.4502	0.7251
T7p	Middle	G3	4	3.6825	2.7318	1.3659	1.092	0.7524	0.3762
T7p	Middle	G4	4	2.5254	2.6044	1.3022	0.607	0.8544	0.4272
T7p	Middle	G5	4	2.3933	2.5135	1.2568	0.417	1.1611	0.5806
T7p	Old	G1	8	8.6271	15.933	5.633	0.315	2.6261	0.9285
T7p	Old	G2	8	49.592	126.11	44.587	1.39	2.3608	0.8347
T7p	Old	G3	8	5.7751	11.09	3.9209	0.19	2.2791	0.8058
T7p	Old	G4	8	2.0915	2.9784	1.053	-0.24	1.6638	0.5882
T7p	Old	G5	8	114.39	280.89	99.31	0.605	3.5218	1.2451
T7p	Young	G1	6	2.2702	4.139	1.6897	-0.14	1.3164	0.5374
T7p	Young	G2	6	8.425	17.203	7.0229	0.566	1.7988	0.7343
T7p	Young	G3	6	0.858	0.4907	0.2003	-0.36	0.7884	0.3219
T7p	Young	G4	6	0.9058	0.4909	0.2004	-0.24	0.5986	0.2444
T7p	Young	G5	6	3.7538	7.0146	2.8637	0.066	1.7742	0.7243
T8m	Middle	G1	4	1.0465	0.8372	0.4186	-0.36	1.2336	0.6168
T8m	Middle	G2	4	4.2651	3.3483	1.6741	0.621	2.1295	1.0648
T8m	Middle	G3	4	4.6722	5.7161	2.858	0.455	2.2906	1.1453
T8m	Middle	G4	4	1.002	1.1753	0.5877	-0.87	1.8328	0.9164
T8m	Middle	G5	4	2.3847	3.6441	1.822	-0.51	2.3299	1.165
T8m	Old	G1	8	1.8621	1.9957	0.7056	0.175	1.0014	0.354
T8m	Old	G2	8	3.6468	3.9372	1.392	-0.51	3.0764	1.0877
T8m	Old	G3	8	28.172	72.771	25.729	-0.81	4.5469	1.6076
T8m	Old	G4	8	29.404	76.656	27.102	0.184	3.2735	1.1573
T8m	Old	G5	8	14.569	37.326	13.197	-0.22	3.0152	1.066
T8m	Young	G1	6	2.4065	1.8751	0.7655	-0.09	2.363	0.9647
T8m	Young	G2	6	1.4699	1.3092	0.5345	-0.66	2.3045	0.9408
T8m	Young	G3	6	1.1874	1.143	0.4666	-0.82	2.3078	0.9421
T8m	Young	G4	6	0.7452	0.7102	0.2899	-1.27	2.1725	0.8869
T8m	Young	G5	6	2.6568	3.1499	1.286	0.029	1.7187	0.7016

T8p	Middle	G1	4	2.433	1.9244	0.9622	0.586	0.967	0.4835
T8p	Middle	G2	4	4.2684	6.0273	3.0137	0.756	1.2873	0.6436
T8p	Middle	G3	4	10.44	7.7946	3.8973	2.123	0.7975	0.3987
T8p	Middle	G4	4	1.7434	1.724	0.862	0.255	0.8378	0.4189
T8p	Middle	G5	4	5.3354	9.6273	4.8136	0.078	2.0653	1.0326
T8p	Old	G1	8	5.6282	8.214	2.9041	0.709	1.6513	0.5838
T8p	Old	G2	8	41.576	111.02	39.252	1.034	2.1323	0.7539
T8p	Old	G3	8	782.55	2201.1	778.2	1.919	2.963	1.0476
T8p	Old	G4	8	4.5177	6.6334	2.3453	0.633	1.5163	0.5361
T8p	Old	G5	8	31.998	77.482	27.394	0.759	2.3372	0.8263
T8p	Young	G1	6	1.83	2.4954	1.0188	-0.83	2.2424	0.9154
T8p	Young	G2	6	0.5121	0.4136	0.1688	-1.49	1.9427	0.7931
T8p	Young	G3	6	0.5765	0.3981	0.1625	-1.15	1.6862	0.6884
T8p	Young	G4	6	1.1134	1.3909	0.5678	-1.05	2.3114	0.9436
T8p	Young	G5	6	0.6732	0.5319	0.2171	-1.01	1.5308	0.6249
T9m	Middle	G1	4	3.3886	3.6082	1.8041	0.398	1.8094	0.9047
T9m	Middle	G2	4	5.6796	6.5639	3.2819	0.77	2.0622	1.0311
T9m	Middle	G3	4	5.9089	6.6743	3.3372	0.784	2.1949	1.0975
T9m	Middle	G4	4	0.8646	0.6881	0.3441	-0.64	1.4106	0.7053
T9m	Middle	G5	4	7.5983	5.6894	2.8447	1.243	2.0803	1.0402
T9m	Old	G1	8	2.0821	1.9997	0.707	0.144	1.4263	0.5043
T9m	Old	G2	8	4.1108	5.644	1.9955	-0.08	2.2807	0.8064
T9m	Old	G3	8	4.625	10.196	3.6047	-0.2	2.0619	0.729
T9m	Old	G4	8	5.2745	12.442	4.3989	-0.46	2.3532	0.832
T9m	Old	G5	8	1.961	3.1735	1.122	-0.16	1.3284	0.4697
T9m	Young	G1	6	1.4183	1.6875	0.6889	-0.36	1.5726	0.642
T9m	Young	G2	6	1.6956	1.4186	0.5791	-0.71	2.5036	1.0221
T9m	Young	G3	6	1.1232	1.1572	0.4724	-0.97	2.1096	0.8612
T9m	Young	G4	6	2.4256	4.3129	1.7607	-0.07	1.355	0.5532
T9m	Young	G5	6	3.3318	4.9496	2.0207	-0.18	2.0903	0.8534
T9p	Middle	G1	4	2.6681	3.0022	1.5011	0.475	1.1701	0.5851
T9p	Middle	G2	4	4.7105	5.7533	2.8767	0.844	1.4534	0.7267
T9p	Middle	G3	4	5.591	5.3328	2.6664	1.336	1.0401	0.5201
T9p	Middle	G4	4	4.113	6.2473	3.1236	0.625	1.3348	0.6674
T9p	Middle	G5	4	3.7173	5.8541	2.9271	0.145	1.826	0.913
T9p	Old	G1	8	15.86	21.659	7.6576	0.655	2.8929	1.0228
T9p	Old	G2	8	90.754	240.22	84.93	1.457	2.6993	0.9544
T9p	Old	G3	8	230.55	642.41	227.13	0.944	3.055	1.0801
T9p	Old	G4	8	8.0346	13.538	4.7863	0.33	2.6724	0.9448
T9p	Old	G5	8	25.797	68.815	24.33	0.144	2.5202	0.891
T9p	Young	G1	6	1.1306	2.3697	0.9674	-1.64	2	0.8165
T9p	Young	G2	6	0.4911	0.5266	0.215	-1.59	1.8491	0.7549
T9p	Young	G3	6	1.1929	2.3028	0.9401	-1.37	2.104	0.8589

T9p	Young	G4	6	0.3334	0.3514	0.1435	-2.04	1.9112	0.7803
T9p	Young	G5	6	0.5787	0.399	0.1629	-0.92	1.1909	0.4862

### APPENDIX III: PEARSON CORRELATION COEFFICIENTS BETWEEN THE TEN GENES

#### KEY: GENES (T)

T1; AMPK $\alpha$ 1,

T2; AMPK $\alpha$ 2,

T3; AMPK $\beta$ 1,

T4; AMPK $\beta$ 2,

T5; AMPK $\gamma$ 1,

T6; AMPK $\gamma$ 1,

T7; AMPK $\gamma$ 3,

T8; IGF-1,

T9; GLUT4

T10; IR

#### KEY: Treatments (G)

G0: Control

G1: Insulin

G2: human chorionic gonadotropin hCG

G3: EGF

G4: IGF-1

G5: Progesterone

#### KEY: Media

m: minus (without FBS and glucose)

p: plus (without FBS and glucose).

Table 1

Pearson Correlation Coefficients Prob >  r  under H0: Rho=0 Number of Observations										
	T1m	T2m	T3m	T4m	T5m	T6m	T7m	T8m	T9m	T10m
<b>T1m</b>	1.00000	0.73377	0.77235	0.27550	0.22784	-0.05782	0.48700	0.00548	-0.11508	0.31845
T1m		<.0001	<.0001	0.0088	0.0495	0.8094	<.0001	0.9593	0.2802	0.0022
	90	90	90	90	75	81	90	90	90	90
<b>T2m</b>	0.73377	1.00000	0.78332	0.44745	0.50988	-0.02559	0.80648	0.22148	0.02922	0.53143
T2m	<.0001		<.0001	<.0001	<.0001	0.8208	<.0001	0.0359	0.7845	<.0001
	90	90	90	90	75	81	90	90	90	90
<b>T3m</b>	0.77235	0.78332	1.00000	0.34888	0.18373	-0.05782	0.51199	0.07477	-0.03717	0.34387
T3m	<.0001	<.0001		0.0008	0.1148	0.8094	<.0001	0.4837	0.7280	0.0009
	90	90	90	90	75	81	90	90	90	90
<b>T4m</b>	0.27550	0.44745	0.34888	1.00000	0.46773	-0.05309	0.50283	0.52865	0.42282	0.58801
T4m	0.0088	<.0001	0.0008		<.0001	0.8379	<.0001	<.0001	<.0001	<.0001
	90	90	90	90	75	81	90	90	90	90
<b>T5m</b>	0.22784	0.50988	0.18373	0.46773	1.00000	0.31931	0.63720	0.27575	0.10842	0.43301
T5m	0.0495	<.0001	0.1148	<.0001		0.0052	<.0001	0.0168	0.3545	0.0001
	75	75	75	75	75	75	75	75	75	75
<b>T6m</b>	-0.05782	-0.02559	-0.05782	-0.05309	0.31931	1.00000	0.02028	-0.01931	-0.04473	-0.04153
T6m	0.8094	0.8208	0.8094	0.8379	0.0052		0.8575	0.8841	0.6917	0.7128
	81	81	81	81	75	81	81	81	81	81
<b>T7m</b>	0.48700	0.80648	0.51199	0.50283	0.63720	0.02028	1.00000	0.27111	0.15011	0.57705
T7m	<.0001	<.0001	<.0001	<.0001	<.0001	0.8575		0.0097	0.1579	<.0001
	90	90	90	90	75	81	90	90	90	90
<b>T8m</b>	0.00548	0.22148	0.07477	0.52865	0.27575	-0.01931	0.27111	1.00000	0.78404	0.79827
T8m	0.9593	0.0359	0.4837	<.0001	0.0168	0.8841	0.0097		<.0001	<.0001
	90	90	90	90	75	81	90	90	90	90
<b>T9m</b>	-0.11508	0.02922	-0.03717	0.42282	0.10842	-0.04473	0.15011	0.78404	1.00000	0.58780
T9m	0.2802	0.7845	0.7280	<.0001	0.3545	0.6917	0.1579	<.0001		<.0001
	90	90	90	90	75	81	90	90	90	90
<b>T10m</b>	0.31845	0.53143	0.34387	0.58801	0.43301	-0.04153	0.57705	0.79827	0.58780	1.00000
T10m	0.0022	<.0001	0.0009	<.0001	0.0001	0.7128	<.0001	<.0001	<.0001	
	90	90	90	90	75	81	90	90	90	90

Table 2

Pearson Correlation Coefficients Prob >  r  under H0: Rho=0 Number of Observations										
	T1p	T2p	T3p	T4p	T5p	T6p	T7p	T8p	T9p	T10p
<b>T1p</b>	1.00000	0.38860	0.83766	0.92814	-0.02415	0.01598	-0.02298	-0.02894	-0.03922	-0.05858
T1p		0.0002	<.0001	<.0001	0.8382	0.8896	0.8307	0.7878	0.7152	0.5877
	89	89	89	89	74	78	89	89	89	88
<b>T2p</b>	0.38860	1.00000	0.58060	0.42936	-0.01947	0.12043	0.00163	-0.05315	-0.06948	-0.07137
T2p	0.0002		<.0001	<.0001	0.8683	0.2904	0.9879	0.6188	0.5153	0.5063
	89	90	90	90	75	79	90	90	90	89
<b>T3p</b>	0.83766	0.58060	1.00000	0.92627	0.07476	0.05748	-0.03578	0.00333	-0.00734	-0.02469
T3p	<.0001	<.0001		<.0001	0.5238	0.6148	0.7378	0.9751	0.9452	0.8183
	89	90	90	90	75	79	90	90	90	89
<b>T4p</b>	0.92814	0.42936	0.92627	1.00000	0.01329	-0.00330	-0.02072	-0.03383	-0.04214	-0.04958
T4p	<.0001	<.0001	<.0001		0.9099	0.9770	0.8463	0.7516	0.6933	0.6445
	89	90	90	90	75	79	90	90	90	89
<b>T5p</b>	-0.02415	-0.01947	0.07476	0.01329	1.00000	0.08403	0.06452	0.72275	0.82873	0.87919
T5p	0.8382	0.8683	0.5238	0.9099		0.4766	0.5823	<.0001	<.0001	<.0001
	74	75	75	75	75	74	75	75	75	74
<b>T6p</b>	0.01598	0.12043	0.05748	-0.00330	0.08403	1.00000	-0.02109	0.05561	0.05032	0.04410
T6p	0.8896	0.2904	0.6148	0.9770	0.4766		0.8536	0.6265	0.6596	0.7014
	78	79	79	79	74	79	79	79	79	78
<b>T7p</b>	-0.02298	0.00163	-0.03578	-0.02072	0.06452	-0.02109	1.00000	0.02005	0.02473	0.34127
T7p	0.8307	0.9879	0.7378	0.8463	0.5823	0.8536		0.8512	0.8170	0.0011
	89	90	90	90	75	79	90	90	90	89
<b>T8p</b>	-0.02894	-0.05315	0.00333	-0.03383	0.72275	0.05561	0.02005	1.00000	0.94989	0.65449
T8p	0.7878	0.6188	0.9751	0.7516	<.0001	0.6265	0.8512		<.0001	<.0001
	89	90	90	90	75	79	90	90	90	89
<b>T9p</b>	-0.03922	-0.06948	-0.00734	-0.04214	0.82873	0.05032	0.02473	0.94989	1.00000	0.81902
T9p	0.7152	0.5153	0.9452	0.6933	<.0001	0.6596	0.8170	<.0001		<.0001
	89	90	90	90	75	79	90	90	90	89
<b>T10p</b>	-0.05858	-0.07137	-0.02469	-0.04958	0.87919	0.04410	0.34127	0.65449	0.81902	1.00000
T10p	0.5877	0.5063	0.8183	0.6445	<.0001	0.7014	0.0011	<.0001	<.0001	
	88	89	89	89	74	78	89	89	89	89

Table 3

Spearman Correlation Coefficients Prob >  r  under H0: Rho=0 Number of Observations										
	T1m	T2m	T3m	T4m	T5m	T6m	T7m	T8m	T9m	T10m
<b>T1m</b>	1.00000	0.60840	0.73729	0.40201	-0.10343	0.12984	-0.05383	-0.37593	-0.27759	0.14333
T1m		<.0001	<.0001	<.0001	0.3772	0.2480	0.6143	0.0003	0.0081	0.1778
	90	90	90	90	75	81	90	90	90	90
<b>T2m</b>	0.60840	1.00000	0.76519	0.43408	0.05087	0.47706	0.04968	-0.00263	-0.06853	0.34550
T2m	<.0001		<.0001	<.0001	0.6647	<.0001	0.6419	0.9803	0.5210	0.0009
	90	90	90	90	75	81	90	90	90	90
<b>T3m</b>	0.73729	0.76519	1.00000	0.57012	0.06982	0.39199	0.07344	-0.08333	-0.12019	0.40905
T3m	<.0001	<.0001		<.0001	0.5517	0.0003	0.4915	0.4349	0.2592	<.0001
	90	90	90	90	75	81	90	90	90	90
<b>T4m</b>	0.40201	0.43408	0.57012	1.00000	0.07693	0.33173	0.26430	0.30225	0.25968	0.28217
T4m	<.0001	<.0001	<.0001		0.5118	0.0025	0.0118	0.0038	0.0134	0.0071
	90	90	90	90	75	81	90	90	90	90
<b>T5m</b>	-0.10343	0.05087	0.06982	0.07693	1.00000	0.32016	-0.13885	0.37209	0.32290	-0.10216
T5m	0.3772	0.6647	0.5517	0.5118		0.0051	0.2348	0.0010	0.0047	0.3831
	75	75	75	75	75	75	75	75	75	75
<b>T6m</b>	0.12984	0.47706	0.39199	0.33173	0.32016	1.00000	0.17830	0.24471	0.12988	0.10770
T6m	0.2480	<.0001	0.0003	0.0025	0.0051		0.1113	0.0277	0.2478	0.3385
	81	81	81	81	75	81	81	81	81	81
<b>T7m</b>	-0.05383	0.04968	0.07344	0.26430	-0.13885	0.17830	1.00000	0.22206	0.25292	0.31616
T7m	0.6143	0.6419	0.4915	0.0118	0.2348	0.1113		0.0354	0.0162	0.0024
	90	90	90	90	75	81	90	90	90	90
<b>T8m</b>	-0.37593	-0.00263	-0.08333	0.30225	0.37209	0.24471	0.22206	1.00000	0.73697	0.26456
T8m	0.0003	0.9803	0.4349	0.0038	0.0010	0.0277	0.0354		<.0001	0.0117
	90	90	90	90	75	81	90	90	90	90
<b>T9m</b>	-0.27759	-0.06853	-0.12019	0.25968	0.32290	0.12988	0.25292	0.73697	1.00000	0.24121
T9m	0.0081	0.5210	0.2592	0.0134	0.0047	0.2478	0.0162	<.0001		0.0220
	90	90	90	90	75	81	90	90	90	90
<b>T10m</b>	0.14333	0.34550	0.40905	0.28217	-0.10216	0.10770	0.31616	0.26456	0.24121	1.00000
T10m	0.1778	0.0009	<.0001	0.0071	0.3831	0.3385	0.0024	0.0117	0.0220	
	90	90	90	90	75	81	90	90	90	90

**Table:** shows the Spearman Correlation Coefficients between ten genes (T1; AMPK $\alpha$ 1, T2;AMPK $\alpha$ 2,T3; AMPK $\beta$ 1,T4; AMPK $\beta$ 2, T5;AMPK $\gamma$ 1,T6; AMPK $\gamma$ 1, T7;AMPK $\gamma$ 3T8;IGF-1, T9;GLUT4, and T10;IR). Were m; minus (without FBS and glucose).

Table 4

Spearman Correlation Coefficients Prob >  r  under H0: Rho=0 Number of Observations										
	T1p	T2p	T3p	T4p	T5p	T6p	T7p	T8p	T9p	T10p
<b>T1p</b> T1p	1.00000 89	0.63956 <.0001 89	0.77201 <.0001 89	0.72127 <.0001 89	0.40231 0.0004 74	0.56747 <.0001 78	0.12100 0.2587 89	-0.21150 0.0466 89	-0.09778 0.3620 89	0.25888 0.0149 88
<b>T2p</b> T2p	0.63956 <.0001 89	1.00000 90	0.66722 <.0001 90	0.66233 <.0001 90	0.14096 0.2277 75	0.41378 0.0002 79	-0.04534 0.6713 90	-0.43158 <.0001 90	-0.46491 <.0001 90	0.12450 0.2451 89
<b>T3p</b> T3p	0.77201 <.0001 89	0.66722 <.0001 90	1.00000 90	0.67690 <.0001 90	0.46332 <.0001 75	0.58428 <.0001 79	0.08567 0.4221 90	-0.18157 0.0868 90	-0.10943 0.3045 90	0.20320 0.0561 89
<b>T4p</b> T4p	0.72127 <.0001 89	0.66233 <.0001 90	0.67690 <.0001 90	1.00000 90	0.38918 0.0006 75	0.56983 <.0001 79	0.23874 0.0234 90	-0.17346 0.1021 90	-0.15251 0.1513 90	0.12551 0.2412 89
<b>T5p</b> T5p	0.40231 0.0004 74	0.14096 0.2277 75	0.46332 <.0001 75	0.38918 0.0006 75	1.00000 75	0.69022 <.0001 74	0.17522 0.1327 75	0.20495 0.0777 75	0.27042 0.0190 75	0.16868 0.1508 74
<b>T6p</b> T6p	0.56747 <.0001 78	0.41378 0.0002 79	0.58428 <.0001 79	0.56983 <.0001 79	0.69022 <.0001 74	1.00000 79	0.33665 0.0024 79	0.09899 0.3854 79	0.07626 0.5041 79	0.11829 0.3023 78
<b>T7p</b> T7p	0.12100 0.2587 89	-0.04534 0.6713 90	0.08567 0.4221 90	0.23874 0.0234 90	0.17522 0.1327 75	0.33665 0.0024 79	1.00000 90	0.26787 0.0107 90	0.27409 0.0089 90	0.37493 0.0003 89
<b>T8p</b> T8p	-0.21150 0.0466 89	-0.43158 <.0001 90	-0.18157 0.0868 90	-0.17346 0.1021 90	0.20495 0.0777 75	0.09899 0.3854 79	0.26787 0.0107 90	1.00000 90	0.67517 <.0001 90	0.26876 0.0109 89
<b>T9p</b> T9p	-0.09778 0.3620 89	-0.46491 <.0001 90	-0.10943 0.3045 90	-0.15251 0.1513 90	0.27042 0.0190 75	0.07626 0.5041 79	0.27409 0.0089 90	0.67517 <.0001 90	1.00000 90	0.31105 0.0030 89
<b>T10p</b> T10p	0.25888 0.0149 88	0.12450 0.2451 89	0.20320 0.0561 89	0.12551 0.2412 89	0.16868 0.1508 74	0.11829 0.3023 78	0.37493 0.0003 89	0.26876 0.0109 89	0.31105 0.0030 89	1.00000 89

**Table :** shows the Pearson Correlation Coefficients between ten genes (T1; AMPK $\alpha$ 1, T2;AMPK $\alpha$ 2,T3; AMPK $\beta$ 1,T4; AMPK $\beta$ 2, T5;AMPK $\gamma$ 1,T6; AMPK $\gamma$ 1, T7;AMPK $\gamma$ 3, T8;GF-1, T9;GLUT4, and T10;IR). Were P; Plus FBS and glucose. The underlined in red indicate a positive significant correlation between changes in expression of the two genes being compared.

Table 5

Pearson Correlation Coefficients Prob >  r  under H0: Rho=0 Number of Observations										
	logAMPKa1	logAMPKa2	logAMPKB1	logAMPKB2	logAMPKg1	logAMPKg2	logAMPKg3	logIGF1	logGLUT4	logINSR
logAMPKa1	1.00000 170	<u>0.79720</u> <.0001 170	<u>0.87584</u> <.0001 170	<u>0.81928</u> <.0001 170	0.24081 0.0016 170	0.48471 <.0001 170	0.24575 0.0013 169	-0.00014 0.9988 160	-0.05463 0.4792 170	0.28733 0.0001 170
logAMPKa2	0.79720 <.0001 170	1.00000 170	<u>0.82013</u> <.0001 170	<u>0.70994</u> <.0001 170	0.08545 0.2679 170	0.44620 <.0001 170	0.24130 0.0016 169	-0.01848 0.8166 160	-0.20552 0.0072 170	0.34184 <.0001 170
logAMPKB1	0.87584 <.0001 170	0.82013 <.0001 170	1.00000 170	<u>0.75772</u> <.0001 170	0.30279 <.0001 170	0.50412 <.0001 170	0.22883 0.0028 169	0.05629 0.4796 160	-0.07360 0.3401 170	0.35183 <.0001 170
logAMPKB2	0.81928 <.0001 170	0.70994 <.0001 170	0.75772 <.0001 170	1.00000 170	0.25690 0.0007 170	0.49156 <.0001 170	0.40514 <.0001 169	0.19519 0.0134 160	0.12261 0.1112 170	0.25501 0.0008 170
logAMPKg1	0.24081 0.0016 170	0.08545 0.2679 170	0.30279 <.0001 170	0.25690 0.0007 170	1.00000 170	0.45241 <.0001 170	-0.02085 0.7878 169	0.54033 <.0001 160	0.42422 <.0001 170	0.08109 0.2931 170
logAMPKg2	0.48471 <.0001 170	0.44620 <.0001 170	0.50412 <.0001 170	0.49156 <.0001 170	0.45241 <.0001 170	1.00000 170	0.28343 0.0002 169	0.25994 0.0009 160	0.15930 0.0380 170	0.22364 0.0034 170
logAMPKg3	0.24575 0.0013 169	0.24130 0.0016 169	0.22883 0.0028 169	0.40514 <.0001 169	-0.02085 0.7878 169	0.28343 0.0002 169	1.00000 169	0.10571 0.1848 159	0.23077 0.0025 169	0.43081 <.0001 169
logIGF1	-0.00014 0.9988 160	-0.01848 0.8166 160	0.05629 0.4796 160	0.19519 0.0134 160	0.54033 <.0001 160	0.25994 0.0009 160	0.10571 0.1848 159	1.00000 160	<u>0.74532</u> <.0001 160	0.33192 <.0001 160
logGLUT4	-0.05463 0.4792 170	-0.20552 0.0072 170	-0.07360 0.3401 170	0.12261 0.1112 170	0.42422 <.0001 170	0.15930 0.0380 170	0.23077 0.0025 169	0.74532 <.0001 160	1.00000 170	0.32562 <.0001 170
logINSR	0.28733 0.0001 170	0.34184 <.0001 170	0.35183 <.0001 170	0.25501 0.0008 170	0.08109 0.2931 170	0.22364 0.0034 170	0.43081 <.0001 169	0.33192 <.0001 160	0.32562 <.0001 170	1.00000 170