DISSERTATION

EFFECT OF AGING ON METABOLIC STATUS AND HORMONE RESPONSIVENESS IN THE OVARY

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ABSTRACT

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There were significant effects of mares' ages on gene expression in granolosa cells. Most of the effects we noted were on the expression of *AMPK* subunits*,* italics indicating genes rather than gene protein products, although there were significant effects of animal age on *GLUT4* and *IGF-1R* expression as well. In general, increased expression of selected *AMPK* subunits occurred in older animals and, with one exception, in cells pre-treated with medium that did not contain FBS or additional glucose. Although the underlying cause of increased expression of the *AMPK* subunits was not determined in this study, various cellular stresses such as nutritional deprevation can lead to AMPK activation. This may be sufficient in aging animals to drive an increase in *AMPK* subunit expression. In addition to *AMPK* effects, there was a decrease in *IGF-1R* expression in older animals although this occurred only in cells pre-treated with medium containing FBS and additional glucose. There was also decreased expression of *GLUT4* following hCG treatment and, again, this occurred only when cells were pre-treated with FBS and additional glucose. We then evaluated membrane lipid order to determine whether animal age affects the membrane structure of granulosa cells. Membrane lipid structure including formation of raft microdomains may affect signaling by receptors involved in reproductive and metabolic functions. Insulin receptors, IGF-1 receptors and EGF receptors require highly ordered membrane microdomains for signal transduction. In general, plasma membranes from older animals had more ordered membranes, perhaps due to an increase in *in vivo* cholesterol synthesis with age. Finally, we examined hormone responsiveness of granulosa cells from young and old mares fed a normal hay-based diet or antioxidant-enhanced commericial diet to evaluate hormone responsiveness with aging. Mares fed an enhanced antioxidant-rich diet showed increased insulin responsiveness, which suggests that at least some effects of aging, namely reduced insulin responses can be treated with an improved diet.

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DEDICATION

To My Home Country and Family

Mustafa Ali Elramly

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: BACKGROUND AND SIGNIFICANCE

1.1: Effects of aging on reproductive function in humans and mares

The reproductive organs in females show signs of aging earlier than any other functional system or organs in the body (1) leading to clear differences between reproductive function in younger and older premenopausal women (2,3). With aging in females, there is overall shortening of the estrus cycle compared with young females (4) and fertility begins to diminish as early as ages 30-40 years of age. However, an ovulatory cycles producing oocytes continues until cessation at around 50 years (5-8). In most women, menopause occurs between age 50 and 55 when circulating follicle stimulating hormone (FSH) concentrations becomes higher than those seen in younger women (8,9).Hormone levels of luteinizing hormone (LH), FSH, estradiol (E2), and progesterone (P4) also change through the inter-menstrual interval with increasing age. During pre-menopause, blood levels of FSH and LH increase 3-5 years before cessation of menstruation (5). In women, normal menstrual cycle are 28 days between the ages 25 and 40 and the cycle length gradually shortens until menopause (8,9). Changes in cycle length appear to be due to changes in follicular maturation. Increased levels of FSH and decreased estradiol levels throughout the cycle accompany a shorter follicular phase in premenopausal women. There are also oscillations between short cycles and longer cycles where there is no evidence of ovulation as indicated by corpus luteum formation. With aging, follicles contain fewer granulosa cells and these cells become less responsive to LH (10).

Because of similarities between reproductive function in young and old mares and humans, the equine model is a useful animal model to study reproductive aging (7,11-13). In both mares and women, granulosa and theca cells are important in follicle maturation which is regulated by steroids and growth factors (14). The effects of aging on follicular maturation are mediated through a combination of reproductive hormones including LH, FSH, and cell-secreted hormones such as epidermal growth factor (EGF) that act on the oocyte and follicular granulosa cells (15,16). While responsiveness to gonadotropins by bovine granulosa cells requires insulin and insulin-like growth factor (IGF-1 (17)), decreased embryo viability and increased apoptosis can occur when cells are exposed to high levels of IGF-1 (18).

Aging mares and women exhibit similarities in morphologic and regulatory aspects of follicle dynamics (19). As in women, equine gonadotropins LH and FSH, secreted by the anterior pituitary gland, bind their respective receptors on granulosa cells in the follicle. These receptors are activated by their ligand to yield follicle development and production of cell-secreted factors including EGF-like hormones that act locally on the oocyte or on cumulus cells associated with the oocyte (12). Aging in mares and women is associated with increases in both circulating FSH and LH as well as changes in the length of the estrous cycle and reduced fertility.

At any age, fertility in mares and women can be affected by changes in their metabolic status including reduced insulin sensitivity. Granulosa cell functions are enhanced in the presence of insulin and IGF-1 due to increased responsiveness to gonadotropin by bovine granulosa cells (8). The appearance of aberrant reproductive cycles is often associated with obesity. In humans, insulin resistance and obesity are associated with polycystic ovarian syndrome (PCOS), a major cause of infertility in women (20-22). Abnormally high concentrations of IGF-1 lead to decreased embryo viability and increased apoptosis (18).

Our goal in these studies was to examine the relationship between gene expression and membrane lipid organization in granulosa cells from young and older mares. In additional, we measured intracellular cAMP levels to assess signaling in granulosa cells from young and old mares. We hypothesized that patterns of gene expression by granulosa cells change with aging and that lipid organization of granulosa cells membranes is affected by changes in metabolic function. Both expression of critical receptors and membrane proteins by granulosa cells and changes in membrane lipid structure contributed to altered cell signaling in response to hormones needed for granulosa cell function.

1.2: Ovarian structure

The ovaries are indispensable for female reproduction. There are two ovaries in the lateral wall of the pelvis in a region called the ovarian fossa. Each ovary undergoes structural and functional changes during the reproductive cycle and, depending on the ovarian cycle stage, contains the tunica albuginea, the cortex of which in mares lies inside the medulla (**Figure 1.1**). Ovarian parenchyma contains corpora lutea, medulla, primordial follicles, secondary follicles, tertiary follicles, mature follicles (Graafian follicles), and atretic follicles. There is sequential development of a primary follicle that undergoes ovulation during every ovarian cycle. Primordial follicles consist of single layer of flattened epithelial cells surrounding the oocyte (23). In each ovarian cycle, only one of these primordial follicles typically reaches maturity and ruptures while the remaining follicles undergo atresia (24,25).

Enlargement of the granulosa layer accompanies development of an outer layer derived from thecal stroma called the theca externa. Granulosa cells and theca externa communicate via a fine plexus of vessels in the theca interna. In both mares and women, the theca interna and granulosa cells are sites of steroid hormone production. The growth of the follicle and oocyte is under endocrine control by pituitary gonadotropins (26,27).

The oocytes are surrounded by cumulus granulosa cells which support and provide nutrients for development of oocytes (28). Early in follicular development, the immature oocytes are in contact with granulosa cells. Later in development, a substance containing polysaccharides called the zona pellucida will separate the membranes of oocyte and granulosa cells. After ovulation, the corpus luteum is the endocrine gland that normally develops from cellular components of the ovarian follicle including granulosa and theca cells (29).

1.3: Follicular development and maturation

Folliculogenesis in mares and women starts with the recruitment of a primordial follicle, development of secondary and tertiary follicles and finally formation of mature Graafian follicles. Folliculogenesis ends in either ovulation or follicular death by atresia. In both mares and women, folliculogenesis can be divided into two phases (30,31). The first phase is the gonadotropin-independent phase, which starts at any time postnatally and continues until puberty. This phase is controlled mainly by locally-produced growth factors such as growth differentiation factor-9 (GDF-9), bone morphogenetic protein-15 (BMP-15), and platelet derived growth factor (PDGF) which promote oocyte growth and are required for primordial follicle activation (32,33). The second phase is dependent on gonadotrophic hormones released from the anterior lobe of the pituitary gland.

During the gonadotropin-dependent phase, there is an increase in size of the follicle regulated by LH and FSH as well as growth factors (34). LH and FSH receptors are structurally very similar and are present in the theca and mural granulosa cell membranes, respectively (35). Under the influence of LH, theca cells produce androgens, which pass through the basal lamina and are aromatized to estrogens by the granulosa cells under the control of FSH. Increased levels of estrogen positively feed back to the anterior pituitary to increase secretion of LH which further increases estrogen production. This increase in estrogen production accompanies the transition from the antral stage to the preovulatory stage of follicular development. Selection of the dominant follicle during follicular deviation is associated with an increase in LH (36,37) and a decline in FSH. Changes in levels of both hormones is necessary for the establishment of deviation (36,38). The LH surge promotes major changes in ovarian preovulatory follicles (39) including terminal differentiation of follicular cells and oocyte maturation. This is followed by ovulation and development of corpus luteum (40,41).

1.4: The effects of aging on cell function

1.4.1: AMPK and metabolic activity in aging

A number of changes in follicular gene expression and function accompanies aging including expression and utilization of AMP-activated protein kinase (AMPK). AMPK is heterotrimeric protein kinase that contains three subunits, with the two alternative alpha catalytic subunits (α1, α2), two alternative beta subunits containing a glycogen-sensing domain (β1, β2), and three alternative gamma subunits (γ1, γ2, γ3) (42,43). AMPK contains two γ exchangeable subunits regulatory sites that bind the activating and inhibitory nucleotides AMP and ATP (44-46).

Aging affects cellular AMP/ATP ratios which increase with aging. AMPK functions as a sensor responding to low ATP levels. Regulating signal pathways is accomplished via positive AMPK activation that replenishes cellular ATP supplies via fatty acid oxidation and autophagy (47,48). Negative regulation of AMPK is achieved through the ATP-consuming cell processes including gluconeogenesis and lipid and protein synthesis. AMPK regulation is accomplished through direct phosphorylation of a number of enzymes (49). AMPK role as a central regulator of energy balance made it a promising drug target for managing metabolic diseases at cellular and whole-body levels such as type II diabetes and obesity (46,50).

Insulin and AMPK can also cooperate to regulate plasma glucose levels. In skeletal muscle, both insulin and AMPK activation stimulate glucose uptake via increased translocation of GLUT4 vesicles to the plasma membrane. (51). 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.

1.4.2: Insulin-mediated signaling and aging

Insulin resistance is one sign of metabolic syndrome (MS) in humans. Clinical signs are obesity, increased body weight, triglyceridemia, dyslipidemia and increased blood pressure. Johnson’s group (52) has observed that obesity is accompanied by other signs of equine metabolic syndrome (EMS) and is associated with an increased risk of other equine diseases including laminitis. The progression of EMS to frank diabetes mellitus in horses is characterized by hyperglycemia, polyuria and increased weight as well as a decrease in insulin or insulin effectiveness (53,54). In additional, obesity is associated with insulin resistance and glucose intolerance. In horses, metabolic syndrome is similar to human metabolic syndrome; both insulin resistance and obesity are associated with the syndrome in both species (52). With the development of diabetes, glucose levels rise to abnormally high levels due to pancreatic failure. Type 2 diabetes is also associated with pituitary pars intermediate dysfunction (PPID). In horses, Type 2 diabetes is probably the more common disease.

The effect of insulin on protein synthesis is mediated in part by activation of the TOR pathway via phosphorylation of TSC2, whereas activation of AMPK causes phosphorylation of different sites on TSC2 and inhibits TOR (55,56). In other cases, insulin and AMPK cooperate, particularly in processes that regulate plasma glucose levels. In skeletal muscle, both insulin and AMPK activation stimulate glucose uptake by increasing translocation of GLUT4 to the plasma membrane. Insulin stimulates cellular growth by activation of the mitogen-activated protein (MAP) kinase extracellular signal-regulated kinase (ERK). This route involves the tyrosine phosphorylation of IRS proteins and/or Shc, which in turn interact with the adapter protein Grb-2 recruiting the son-of-sevenless (SOS) exchange protein to the plasma membrane for activation of Ras. Ras is also activated by stimulation of the tyrosine phosphatase, SHP2, through its interaction with receptor substrates such as Gab-1 or IRS1/2. When activated, Ras stimulates a serine kinase cascade through activation of Raf, MEK, and ERK. Once ERK is activated, it translocates to the nucleus to catalyze the phosphorylation of transcription factors such as p62TCF. This transcriptional event leads to cellular proliferation or differentiation. Inhibition of this pathway by dominant negative mutants or pharmacological inhibitors prevents cell growth by insulin, but without any affect on the metabolic actions of the hormone (57). This explains the complexity of the signaling pathways of insulin that regulate GLUT4 recycling. Winder and Hardie (58) proposed that activators of AMPK could be used to treat Type 2 diabetes.

1.4.3: LH receptor and EGF receptor in granulosa cells

LH activation of its receptor on mural granulosa cells stimulates cAMP signaling which, in turn, induces the expression of the receptor tyrosine kinases involved in signaling cascades used by epidermal growth factors (EGF). Activation of EGFRs can occur in an autocrine and/or paracrine manner within mural layer and cumulus cells (59-62). EGF-like growth factors probably function as mediators of LH action in ovulatory follicles. It is generally accepted that LH regulates mRNA and protein synthesis and that 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 on granulosa and cumulus cells, respectively, to activate the EGF receptor and increase metabolic activity within target cells. Activation of EGF receptor signaling in cumulus cells, together with cAMP priming, triggers oocyte nuclear maturation and acquisition of developmental competence as well as cumulus expansion (59,63).

There are also effects of aging on gene expression. Ashwish identified significant differences in expression of various AMPK subunits between younger and older animals (64) as well as expression of the genes for GLUT4 and IGF-1 receptor. When significant differences in gene expression were observed, older animals typically expressed higher levels of that particular gene.

1.4.4: Assessing hormone responsiveness by measuring intracellular cAMP levels

Ligand binding initiates a conformational change in LH receptors that in turn activates G proteins (65). This change leads to activation of the heterotrimeric G protein, Gs, exchange of GTP for GDP, and s-GTP subunit dissociation from the Gα subunit. The activated Gα subunit interacts with adenylate cyclase (66). Activation of adenylate cyclase by the Gα subunit in turn converts adenosine triphosphate (ATP) to the second messenger molecule, cyclic AMP (cAMP) (67). cAMP binds to the regulatory subunit of PKA and causes activation of PKA subunits (68) which, in turn, initiates a signaling cascade within hormone-responsive cells.

Throughout follicle development, initiation of ovulation and corpus luteum development, binding of LH or FSH to their respective G protein receptors causes an increase in intracellular cAMP. In addition to being an important intracellular second messenger, cAMP has many regulatory effects involving many fundamental biological processes ((69,70); **Figure 1.2**) including effects on cell metabolism, on the cytoskeleton and on transcriptional responses. Intracellular cAMP levels are regulated during lipid metabolism, sugar metabolism and insulin secretion (70,71).

Historically, evaluation of cAMP levels in living cells has relied on biochemical assays. One active area of research that has benefitted from interest in single cell studies is the development of fluorescent sensors, including a cAMP sensor, for real-time monitoring of cell events. Cyclic AMP regulates many cellular functions through its effector proteins including protein kinase A (PKA) and Epac, an exchange protein directly activated by cAMP (72). Guanine nucleotide exchange factors for Rap1 and Rap2 (73) constitute the Epac family of molecules and mediate exchange of GDP for GTP (74). GTPases cycle between an inactive GDP-bound and an active GTP-bound state (**Figure 1.3**).

C-terminal catalytic domain characteristic of exchange factors for Ras family GTPases and an N-terminal regulatory domain constitutes the guanine nucleotide-exchange factor Epac1 (GEF Epac1). The N-terminal regulatory domain contains a cAMP-binding site similar to that of protein kinase A (PKA). It also has a Dishevelled, Egl-10, Pleckstrin (DEP) domain that mediates membrane attachment (73,75). Fluorescent probes detecting intracellular cAMP levels and Epac activation were constructed by Zhang and coworkers (76) by sandwiching full-length Epac1 between CFP and YFP (**Figure 1.4**). In living mammalian cells, elevations in cAMP levels decrease FRET between CFP and YFP and so increase the ratio of cyan-to-yellow emission by 10-30%. Mutating a critical residue responsible for cAMP binding can abolish this response, which can be reversed by removing cAMP-elevating agents (76). After the activation of GPCRs that stimulate Gs proteins, adenylyl cyclases are activated while GPCRs that stimulate Gi proteins are inhibited. Termination of the cAMP effect will then occur via cAMP hydrolysis using a specific phosphodiesterase (77).

1.5: Gene expression with aging

Aging and development of metabolic disease may result in changes in gene expression in response to various ligands as well as changes in metabolic activity. Gene transcription was measured in granulosa cells collected from young and old mares using real-time reverse transcription polymerase chain reaction (RT-qPCR) after the cells were treated with FSH, LH, and EGF, insulin like growth factor-1 (IGF-1), insulin, and progesterone. Also examined were the effects of these ligands on granulosa cells' metabolic activity.

PCR is a method to measure mRNA and cDNA in real time. In reverse transcription PCR (RT-PCR) reactions, the first step of RT-PCR is the synthesis of a DNA/RNA hybrid. RNA is used as a template followed by an additional step to detect and amplify RNA which is reverse-transcribed into complementary DNA (cDNA) using reverse transcriptase. Reverse transcriptase also has an RNase H function, which degrades the RNA portion of the hybrid. The success of RT-PCR relies on quality and purity of the RNA template. The difference between the two reactions is that conventional PCR collects data on the reactions at the end-point, whereas Real Time PCR collects data during its exponential phase.

Real-time RT-PCR uses fluorescence dyes (e.g. SYBR Green I) and is currently the most sensitive and precise method for investigating RNA levels. It has been widely used for absolute and relative quantitation of cellular mRNA. The highly sensitive method allows measurement of different types of RNA levels in the cell based on the kinetics of the amplification of the corresponding double-stranded cDNA. Upon its binding to the minor groove of the double-stranded DNA, SYBR Green I dye increases its fluorescence threshold about 100-fold, and such an increase can be recorded in the very early cycles of amplification. As real-time RT-PCR cycles proceed, the level of amplified DNA is measured after every cycle of amplification. This allows quantification at early cycles when amplification curve has not yet reached its "plateau" range and corresponds to the exponential increase in amounts of DNA.

The general assumption in RT-PCR studies is that each normal cell has a standard expression of housekeeping genes, which work as internal gene controls. It is assumed here that changes to the level of the expression profile for housekeeping genes indicates an abnormality at the genetic level.

1.6: Effects of aging on granulosa cell plasma membranes

The plasma membrane of a cell is a complex structure. The basic structure of the membrane is a fluid bilayer in which proteins are embedded. These proteins may be partly integrated or peripherally attached to the membrane and can have sugar moieties on the outer surface of the protein. Proteins help to transport large molecules, like glucose, across the cell membrane. Some proteins on the cell surface function as receptors for low molecular weight ligands. The plasma membrane forms a selectively permeable layer around the cell with proteins in the bilayer permitting only specific substances to cross the membrane under controlled conditions.

In the fluid mosaic model of the plasma membrane, both lipids and proteins diffuse laterally in the plane of the membrane at rates dependent on the viscosity of the lipid bilayer of cell membrane. Although it is less obvious, lipids also move within the lipid bilayer and in the process make the membrane more fluid. The composition of a membrane can affect lipid fluidity and the motions of lipids and proteins within the bilayer. The membrane phospholipids incorporate fatty acids of varying length and saturation. Unsaturated lipid chains containing carbon-carbon double bonds are more fluid than lipids with saturated fatty acids which have only carbon-carbon single bonds. On the molecular level, unsaturated double bonds make it harder for the lipids to pack together due to kinks in the fatty acid chains. When unsaturated fatty acids increase as a relative percentage of the total membrane lipid, membrane fluidity increases. Membranes composed of lipids with lower melting points require less thermal energy to achieve the same level of fluidity as membranes formed from saturated lipids.

The cholesterol content in a membrane also affects the membrane's structure. Cholesterol is a sterol that helpe regulate molecules entering and exiting the cells. Cholesterol molecules are randomly distributed in the phospholipid bilayer. Cholesterol acts as a "bidirectional" regulator of membrane fluidity. At high temperatures, cholesterol stabilizes the membrane and raises its melting point. At low temperatures, cholesterol intercalates between the phospholipids and prevents them from packing closely. Importantly, unsaturated fatty acids decrease and the content of cholesterol in plasma membranes increase with aging (78).

Plasma membranes are heterogeneous structures. Glycolipids and sphingomyelin are restricted to the outer leaflet of the bilayer, whereas cholesterol and phospholipids are in both leaflets. The association of cholesterol with sphingolipids promotes phase separation apparently because of favorable packing interactions between saturated lipids and sterol (79,80). These concentrated regions containing cholesterol and sphingolipids make up “lipid rafts”, low density microdomains that float in the shorter, unsaturated phospholipids of the bulk membrane (81).

Hormone signaling via plasma membrane receptors requires specialized lipid environments such as rafts. Membrane lipid structure including formation of raft microdomains may affect signaling by receptors involved in reproductive and metabolic functions. Hormone activation of plasma membrane receptors is necessary for follicle development and production of cell-secreted factors including EGF. Gonadotropin responsiveness by bovine granulosa cells is enhanced in the presence of insulin and IGF-1 that act locally on the oocyte or on cumulus cells associated with the oocyte (82,83). LH receptors and EGF receptors require highly ordered membrane microdomains for signal transduction.

1.7: Assessing lipid order in membranes of viable cells

Membrane fluidity is a complex property influenced by biophysical and biochemical factors such as protein composition, phospholipid ratios, cholesterol ratios, and the degree of fatty acid saturation. Changes in membrane composition and structure can alter the conformation and function of transmembrane ion channels, as well as affect the interaction of receptors and effectors, leading to altered signal transduction. Because signaling is impaired with aging and with metabolic disorders, we evaluated membrane lipid packing to determine whether the lipid composition of the granulosa cell membrane changes with aging.

In this project, we evaluated the extent of lipid order in granulosa cells from young and older mares. The overall question we addressed is whether the lipid milieu in the plasma membrane of granulosa cells changes with aging. These studies used Di-4-ANPPDHQ (amino-naphthylethenylpyridinium), an environmentally sensitive lipid probe that differentiates liquid-ordered phase from lipid-disordered phase. It has a useful signal-to-noise ratio and is not internalized readily (84). Jin and coworkers (83) have shown that the probe is sensitive to differences in lipid packing within membrane microdomains where local cholesterol concentrations are high and lipid packing affects the dye's emission spectrum. When excited by a 588 nm source, Di-4-ANPPDHQ undergoes a spectral shift in fluorescence emission to lower wavelengths in the presence of cholesterol and/or in more ordered lipid environments. In disordered lipid environments, the probe emits with emission maxima at 628 nm and shifts to an emission maxima of 588 nm emission in cholesterol-enriched lipid environments (83).

1.8: Research goals

In this project, we used the RT-qPCR quantification of gene expression to examine granulosa cell responses to various ligands including FSH, LH, EGF, IGF-1 and progesterone. We examined the effects of these ligands on expression of the insulin receptor and IGF-1 receptor genes, on expression of the glucose transporter GLUT4 that is up-regulated by insulin and on the expression of subunits for AMPK, an enzyme that reflects the metabolic activity of granulosa cell. We also examined membrane lipid order to determine whether the order of membrane lipids, sensitive to phospholipid content and cholesterol content, changes with aging. One possible cause of reduced fertility with age is a change in highly ordered lipid compartments, particularly in insulin-resistant animals. Finally, we measured hormone responsiveness of granulosa cells from older and younger animals to determine whether aging or an antioxidant-rich diet have effects on, for example, insulin, IGF-1 or EGF-mediated signaling.

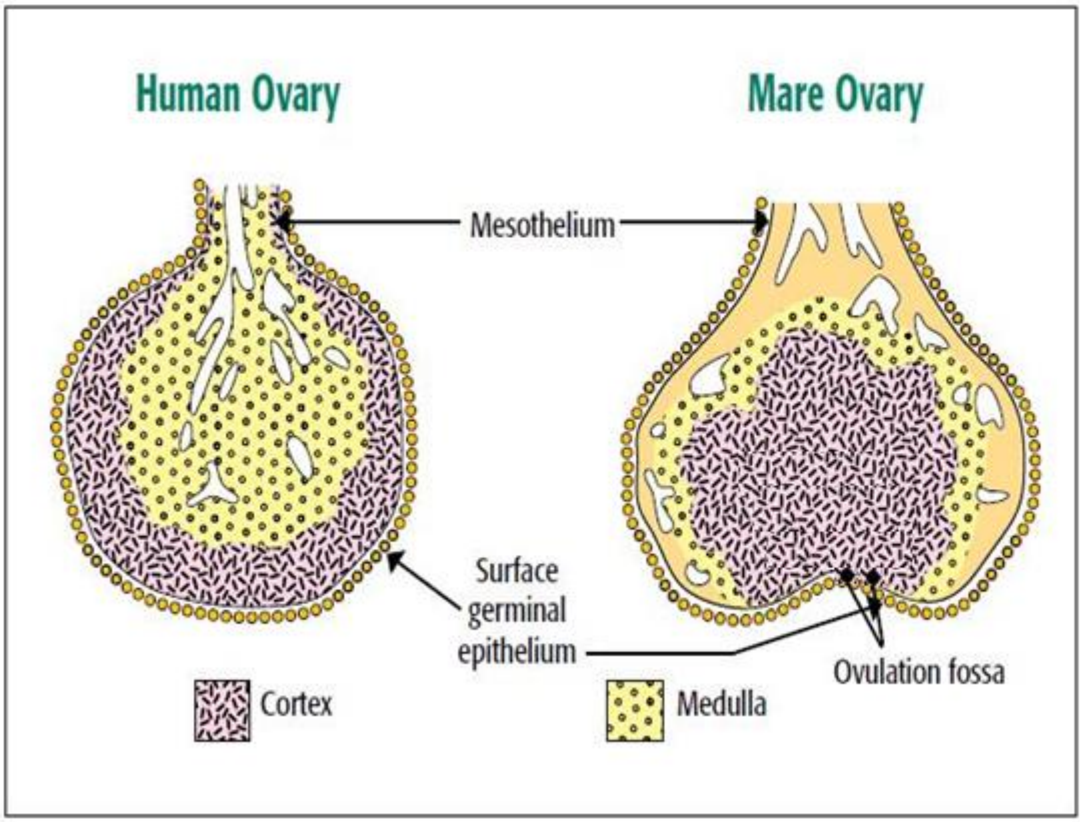


Figure 1.1: Comparation of the human and mare ovary (85).

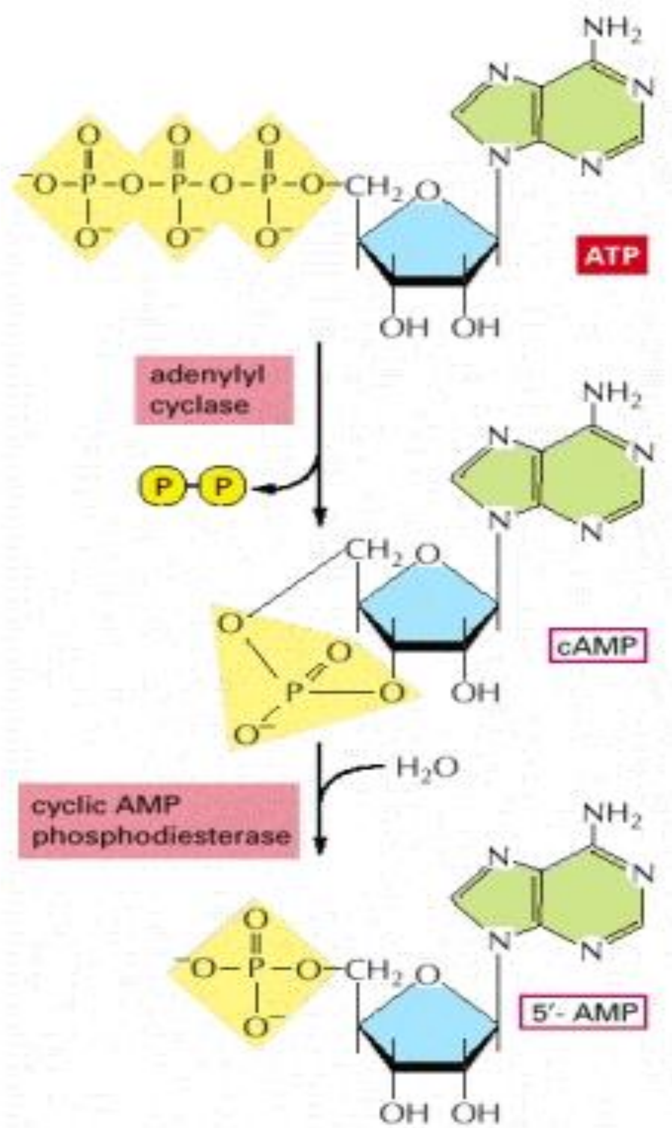


Figure 1.2: The chemical structure of the cAMP second messenger (86).

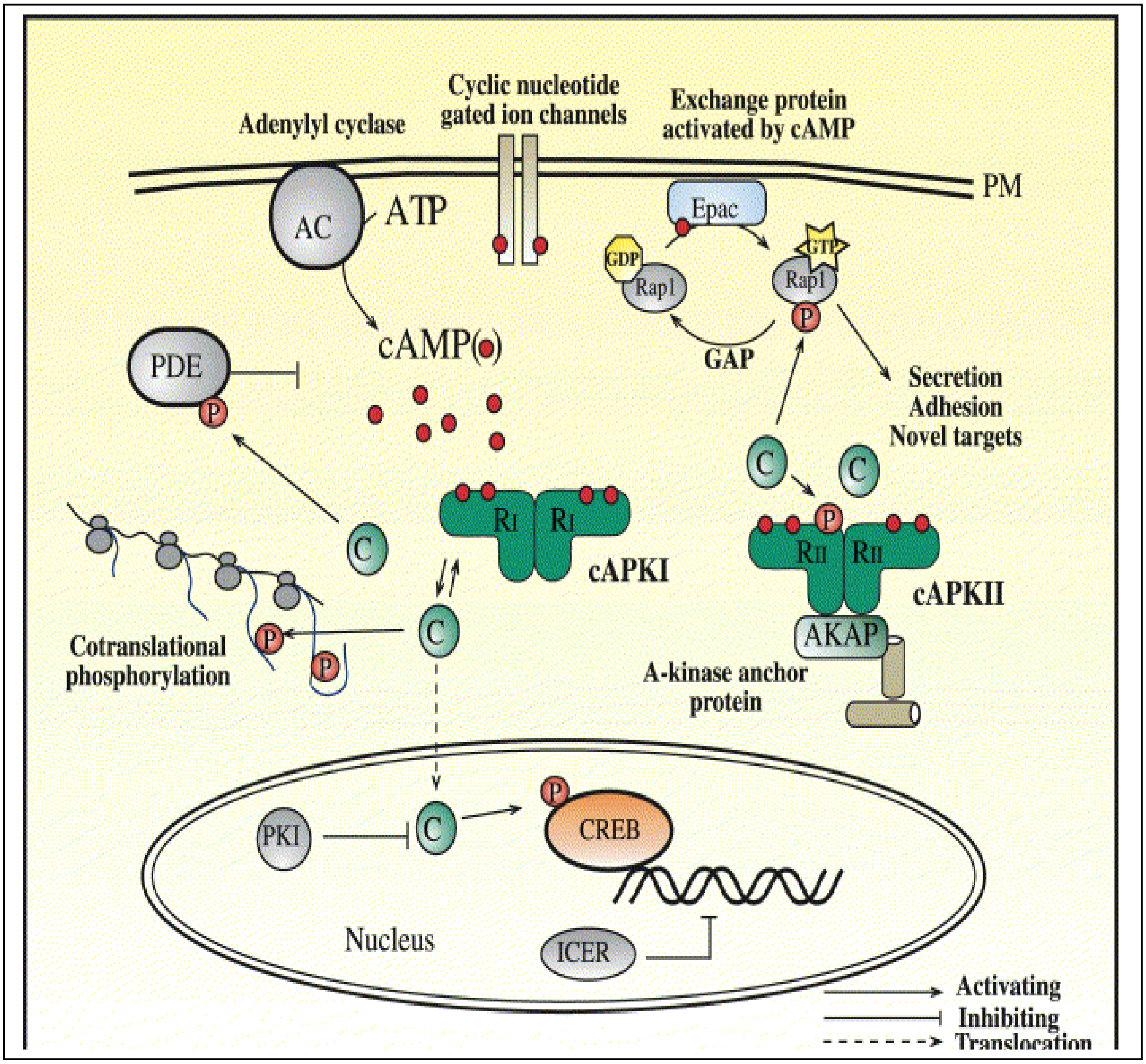


Figure 1.3. Epac is an exchange protein activated by cAMP. This molecule can be targeted to the plasma membrane using a membrane-targeting amino acid sequence and modified to include CFP and YFP. In the absence of cAMP, these fluorophores are close to one another and energy transfer from CFP to YFP occurs. When cAMP binds to Epac, a conformational change in Epac results in physical separation of CFP and YFP and a reduction in energy transfer efficiency (from (87)).

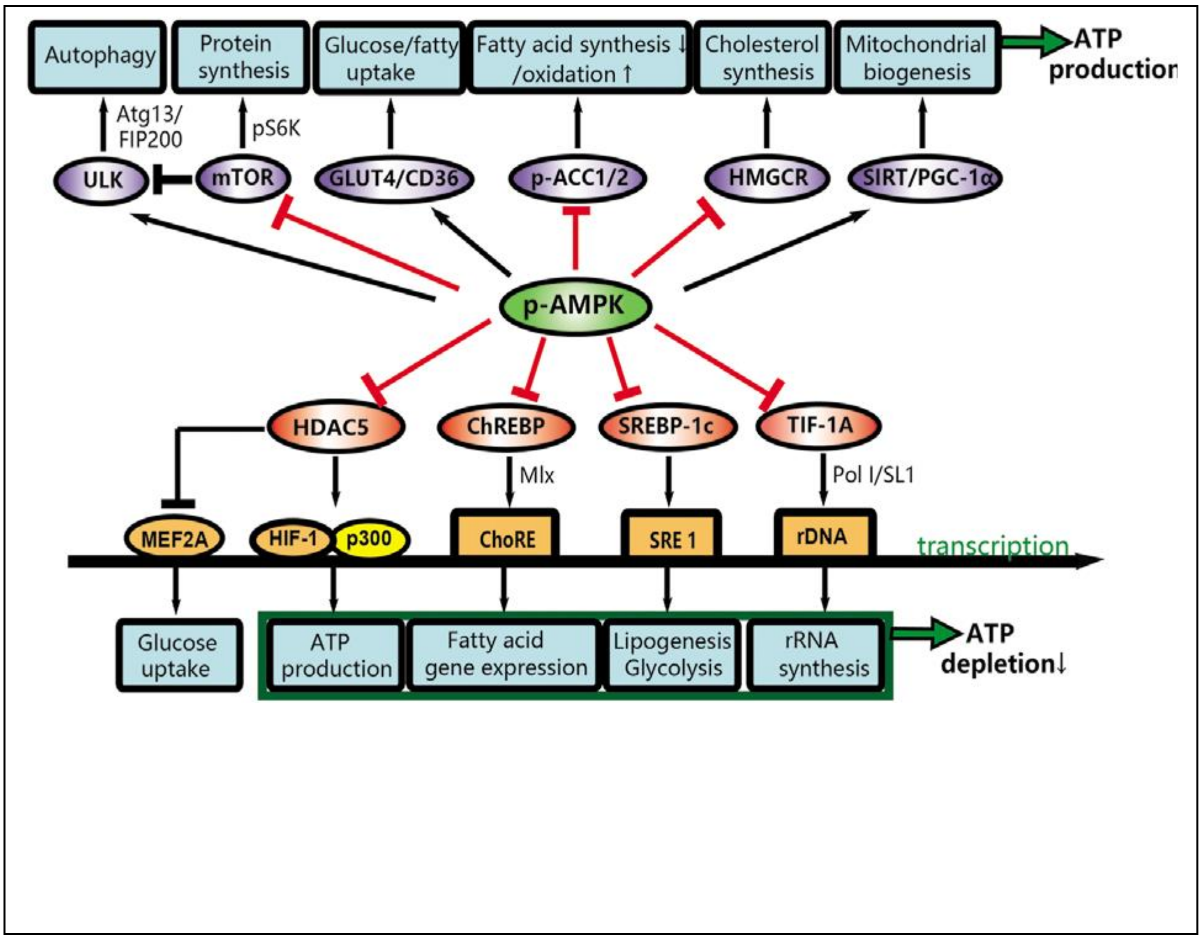


Figure 1.4 AMPK conserves ATP by switching off biosynthetic pathways. Upward arrows are the pathways that AMPK uses to promote ATP production. Downward arrows are the pathways that AMPK used to suppress ATP depletion (from (47)).

CHAPTER 2. MATERIALS AND METHODS

2.1: EVALUATING METABOLIC ACTIVITY IN INDIVIDUAL GRANULOSA CELLS USING RT-PCR

We previously used real-time reverse transcription polymerase chain reaction (RT-PCR) to evaluate gene expression in granulosa cells (64). In this project, data has been re-analyzed in order to evaluate difference in gene expression between younger (≤ than 15 years old) and older mares (> than 15 years old). Response to various ligands in gene expression as well as changes in metabolic activity may result from aging. We used RT-PCR to quantify gene expression in granulosa cells in response to FSH, LH, and EGF, IGF-1, insulin and progesterone. We also examined expression of glucose transporter type 4 (GLUT4), a glucose transporter that is up-regulated by insulin, and the levels of adenosine monophosphate-activated protein kinase (AMPK), which reflects the metabolic activity of granulosa cells.

2.1.1: Cell culture

Equine granulosa cells were obtained from Dr. Carnevale at the Equine Reproduction Laboratory at Colorado State University. Cells were aspirated from dominant follicles approximately 20-24 hours after induction of follicular maturation, when the dominant follicle was ≥35 mm in diameter. Follicles were aspirated from young (n=10) and older (n=8) estrous mares. 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 24 h later by transvaginal, ultrasound-guided follicular aspirations (88).

Fico/Lite-LymphoH was purchased from Atlanta Biological (Catalog # 140110). Dulbecco's Modification of Eagle's Minimum Medium (DMEM) was purchased from Mediatech, Inc, (Manassas, VA). L-glutamine (200mM) and 10,000 U/mL penicillin G, 10 μg/mL streptomycin and 25 μg/mL Fungizone streptomycin solution were purchased from Gemini Bio-Products (West Sacramento, CA). Fetal bovine serum (FBS) was purchased from Gemini Bio-Products (Woodland, CA). Ethylenediamine tetraacetic acid (EDTA) was purchased from Sigma-Aldrich (St. Louis, MO). Glass bottom cell culture dishes, 35 mm in diameter, were purchased from In Vitro Scientific (Sunnyvale, CA).

2.1.2: Treatments of cells

Granulosa cells were separated from tissues debris and red blood cells using Fico/Lite-LymphoH gradients and Manufacturer's instructions. After removing tissue debris and red blood cells from the follicle aspirate, we cultured the remaining granulosa cells in two media. One group of cells was incubated overnight without fetal bovine serum or additional glucose and the other group of cells was incubated with fetal bovine serum and 30mM glucose. Cell culture medium was replaced by serum-free medium before cells were treated for 1 hr with either human epidermal growth factor (EGF; Sigma-Aldrich, E9644 S/GMA, 1 μM), recombinant human insulin like growth factor-1 (IGF-1; Life Technologies, 10 nM), progesterone (P4; Sigma-Aldrich P0130-25G, 100 nM), insulin (Sigma-Aldrich, I-9278, 100 nM), or human chorionic gonadotropin (hCG: Fitzgerald, 100 nM). Cells were harvested by soaking dishes in 5 mM

EDTA pH 7.4 for 5 minutes, removing the EDTA and washing cells using 1x PBS. Cells were then washed using 15 ml centrifuge tubes and centrifugation at 300 x g for 3 min. This wash was repeated to remove any remaining supernatant. Isolated cells were then used either immediately or snap frozen in liquid nitrogen and stored at -80ºC (89).

2.1.3: Total RNA isolation

Frozen samples were stored at -80ºC. RNA isolation from these samples was accomplished using the RNeasy Mini kit (Qiagen Inc., Valencia, CA, Catalog #74104) according to the Manufacturer's recommendations. DNAse digestion using RNAse-free DNAse treatment (Qiagen Inc., Valencia, CA, Catalog #79254) followed RNA isolation to remove any residual genomic DNA. A spectrophotometer at 260 nm and 280 nm (Nano Drop ND-1000) (Nano Drop Technologies Inc., Rockland, DE) was used to determine the total concentration of purified RNA. An estimate of the purity of isolated RNA was determined by calculating the ratio of readings at 260 nm and 280 nm (A260/A280). Pure RNA has 1.7 or greater ratio of A260/A280. Samples for analysis with a ratio of 2.0 or greater of A260/A280 were stored at -80ºC for analysis (90).

2.1.4: Reverse transcription of mRNA

To obtain cDNA (complementary DNA) from the extracted RNA, the qScript cDNA SuperMix (Quanta Biosciences, Inc, Gaithersburg, MD) cDNA synthesis kit was utilized to transcribe messenger RNA into cDNA. 600 ng of RNA/sample was used. For real time PCR analysis, 5µL of RNA was combined with nuclease-free water and qScript Super Mix. RNA was reverse-transcribed by incubating each sample at 25ºC for 5 minutes, at 42ºC for 30 minutes, and at 28ºC for 5 minutes. The resulting cDNA was used immediately.

2.1.5: Primer design and sequencing

Various online databases and NCBI BLAST were used as previously described (64) to design primers for the following ten equine genes, *AMPKα1, AMPKα2, AMPKβ1, AMPKβ2, AMPKγ1, AMPKγ2, AMPKγ3, IGF-1R, GLUT4, and IR*. Those oligonucleotide primers were purchased from Euro Fins (Huntsville, AL). The primer sequences for the ten genes above and primer sequences for the three housekeeping genes (HK), GAPDH, ACTB, and SADH, have been described previously (64). To guarantee specific target gene amplification, the primer efficiency was verified against a standard curve of pooled samples of equine granulosa cells expressing genes of interest as a positive control. Extraction and purification of DNA of 70bp to 10 kb from standard 2% agarose gel was achieved via a Qiaquick Gel Extraction Kit (Qiagen, Valencia, CA, and Catalog # 28704). Sequencing of specific primers was done to test their fidelity. Any remaining gel was rinsed away with buffer PE and then eluted in nuclease-free water. Then the samples were prepared and sent for sequencing at the Colorado State University Proteomics Facility, Fort Collins, CO.

2.1.6: Real-time PCR quantification of mRNA

Real-time PCR was used to examine the relative expression of ten genes involved in IR and AMPK signaling (*AMPKα1, AMPKα2, AMPKβ1, AMPKβ2, AMPKγ1, AMPKγ2, AMPKγ3, IGF-1R, GLUT4, and IR)* and three genes (*GAPDH, ACTB*, and *SADH)* were used as internal control by RT-PCR. A 10-fold serial dilution series was used to determine amplification efficiencies. The analysis was carried out in 10 µL reactions containing Sso Fast Eva Green Super mix (Bio-Rad, Hercules, and CA), 0.5 µM gene specific forward and reverse primer, and cDNA using Light Cycler® 480 Real-Time PCR System (Roche). Eva Green is a far brighter fluorescent nucleic acid dye than SYBR green and so permits better detection of novel DNA. It has well-characterized binding mechanisms, is not toxic, is easy to handle and is safe for disposal. The dye is stable during storage and under PCR reaction conditions and, moreover, enables visualization of electrophoretically-separated PCR products directly in a UV box without needing another gel stain. These characteristics ensure a high degree of specific amplification due to its ability to bind to and detect any dsDNA generated during amplification. Reaction conditions were as follows: 94ºC for 5 minutes, 40 cycles at 94ºC for 15 seconds, 60ºC for 30 seconds, and 72ºC for 15 seconds, and 72ºC for 3 minutes. A melt curve analysis was performed to confirm amplification of single PCR products.

2.1.7: Data analysis of RT-PCR results using standard methods

The cycle threshold Ct method previously described by Schmittgen and Livak was used (91). We calculated the geometric mean of housekeeping gene expression values for use as a normalization factor. The 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 ΔCt necessary to achieve the same level of fluorescence (92). A formula, $2^{-\Delta CT}$, was used to calculate ΔCt and also to normalize our data. The level of gene expression of a particular gene in a treated sample was compared relative to the level of gene expression of untreated samples. Fold changes between treated and untreated samples was calculated as $2^{-\Delta\Delta Ct}$.

2.1.8: Statistical analysis

Analysis of data from young and older mares was completed using the SAS "Proc.Mixed" procedure. A total of (n =18) horses were included in this study (10 young, 8 old). Separately, a repeated measures analysis was conducted for each gene (10 levels) and control ± FBS and 30mM glucose. The log transformed expression was used to satisfy the assumption of normality. Fixed effects included both ages, treatment (6 levels plus and minus = 12) plus all interactions. Tukey-adjusted pairwise comparisons were performed separately for each gene. In addition, Dr. Hess performed the following tests: **Test 1** gave the ANOVA F-tests for main effects and interactions; **Test 2** gave comparisons of treatments (averaging over Ages) for each gene and +/-FBS/glucose level separately; **Test 3** gave comparisons of ages averaging over treatments for each gene and glucose level separately; **Test 4** gave comparisons of treatments for each gene, +/-FBS/glucose and age separately; **Test 5** gave comparisons of -FBS/-30mM glucose versus plus +FBS/+30mM glucose for each gene, age and treatment separately; and **Test 6** gave comparisons of ages for each gene, +/-FBS/glucose and treatments separately. A summary of Dr. Hess' statistical analysis was included in **Appendix I**.

2.2: EVALUATING cAMP ACTIVITY IN INDIVIDUAL GRANULOSA CELLS

Hormone responsiveness of granulosa cells from younger and older mares was evaluated using ICUE3, a cAMP reporter molecule. Lipofectamine 3000 regent and OPTI-MEM reduced serum medium were purchased from Life Technologies (Carlsbad, CA). The ICUE3 plasmid was kindly provided by Dr. Jin Zhang (Johns Hopkins University, Baltimore, USA). Glass bottom cell culture dishes, 35 mm in diameter, were purchased from In Vitro Scientific (Sunnyvale, CA).

2.2.1: Animal diet

Equine granulosa cells from younger mares (<15 years old) and older mares (> 15 years old) were obtained from Dr. Carnevale at the Equine Reproduction Laboratory at Colorado State University.

In some experiments, mares were housed in adjacent dry lots with sheds, with mineral blocks and water ad libitum. Grass/alfalfa mix hay was fed at approximately 2% body weight daily. Mares were paired by age and were randomly assigned to one of two groups. The control group of mares (CTL) received 0.45 kg of a grain mix of corn, oats and barley (COB, Nutrena) topped with 60 ml of corn oil (Mazola Corn Oil). The second group of mares was fed a combination of feed additives designed to promote equine health and fertility [Equine™ GI (147 g), Potency® (14g daily), Motility Plus® (11.5g daily), Healthy Weight Oil (60 mL) and with the addition of antioxidants (coenzyme Q10, 500 mg; pterostilbene, 500 mg; pyrroloquinoline quinone, 40 mg)]. The oil supplements contained differing ratios of n-3 to n-6 PUFA (corn oil, 1:46 and flax oil, 1:0.3). Diet supplements were fed once daily in individual pens to assure consumption. Samples were collected 8 to 13 weeks after supplementation.

2.2.2: Cell culture and transfection

Cells were aspirated from dominant follicles after induction of follicular maturation, when the dominant follicle was ≥35 mm in diameter. Follicles were aspirated from young and older estrous mares in moderate body condition with weights ranging from 485 to 670 kg were used. All procedures were done in accordance with the Colorado State University Institutional Animal Care and Use Committee. Granulosa cells were grown in a 25 cm^2 cell culture flask in DMEM medium supplemented with 10% FBS, 2mM L-glutamine, and 1% penicillin/streptomycin. All cells were maintained at 5% CO_2 and 37°C in a humidified environment. Before transfection, cells were incubated with 5mM EDTA for 5 min and 1.5mL cells were plated in a 35mm glass bottom petri dish.

2.2.3: Granulosa cells transduction with ICUE3

Granulosa cells were divided into two groups and incubated in -FBS/-glucose or +FBS/+glucose media. At the same time cells were transiently transfected with 0.4 µg of ICUE3 plasmid, a membrane-targeted cAMP reporter (93) using Lipofectamine 3000. Two sterilized micro centrifuge tubes were used each containing 125µL of OPTI-MEM medium. Tube 1 contained 0.4µg of ICUE3 and 5µL Lipofectamine 3000, and Tube 2 contained 7.5µL of Lipofectamine 3000. Tube 1 was added drop wise to Tube 2. After 5 min, the mixture was added to the cells in a 35mm Petri dish containing 1ml OPTI-MEM reduced serum medium. Cells were maintained for 12 hrs. in a humidified incubator in 5% CO_2 at 37ºC. Cell medium was then aspirated from the Petri dishes, cells were gently washed twice and maintained in PBS at pH 7.4 containing 0.1% bovine serum albumin (BSA).

2.2.4: Hormones treatment and cAMP analysis

Granulosa cells were imaged using dual emission wavelength ratio imaging, with images collected using a 1.2 N.A. 63x water objective in a Zeiss Axiovert 200M inverted microscope with an Andor EMCCD camera controlled by MetaFluor software. Emission ratios were obtained using a 436DF20 excitation filter, a 455 DRLP dichroic mirror, and two emission filters, 480DF40 for CFP and 535DF30 for YFP. Cell images were taken with a 60s time interval between frames during acquisition. During experiments, data were collected from untreated cells for several minutes before the PBS solution was removed and replaced with either EGF (1μM), recombinant human IGF-1 (10nM), or insulin (100nM) in PBS. In each experiment, a sequence of images was obtained. All data were analyzed by Image J software. Background corrections of fluorescent images were performed by subtracting the intensity of the background from the emission intensities of fluorescent cells expressing ICUE3 (**Figure 2.1**). Emission ratios (CFP/YFP) were then calculated from corrected CFP emission intensity and corrected fluorescence energy transfer (FRET) to YFP following CFP excitation. An increase in CFP emission with an accompanying decrease in the extent of fluorescence energy transfer to YFP was indicative of an increase in cAMP in response to treatment with either insulin, IGF-1 or EGF.

2.2.5: Assays of cAMP in granulosa cells from older mares fed a diet high in anti-oxidants

In some experiments, granulosa cells were used to determine whether older mares fed an anti-oxidant enriched diet exhibited reductions in insulin resistance and improved responsiveness to insulin, IGF-1 or EGF. Before beginning this diet, non-lactating light horse mares were evaluated for body condition. This involved body condition scoring using the Kentucky Equine Research body condition score chart. Older animals (above 15 years of age) were matched for condition and placed in two groups (n=8 animals/group). One group received the anti-oxidant enriched diet described earlier. The other group was fed 0.45 kg of a grain mix of corn, oats and barley (COB, Nutrena) topped with 60 ml of corn oil (Mazola Corn Oil). We then examined the effects of the same hormones used above on granulosa cells from diet-fed older mares, and older mares fed a standard mixed grass/alfalfa and grain mix containing corn oil.

2.2.6: Statistical analysis

Sum Stats: Summary statistics (mean, SD, se) for each group and treatment.

Test 1: For each treatment, groups were compared. The Slice and Age (or Group) columns indicate the comparison. The Estimate column shows the estimated difference between the mean ratios for each comparison. For Project 2 (Diets), both raw (Raw p) and Tukey adjusted (Adj p) p-values are provided. Comparisons that are significant at the 0.05 level are highlighted in pink.

Test 2: For each group, treatments were compared using Tukey's method. The Slice, Treatment and Treatment columns indicate the comparison. The Estimate column shows the estimated difference between the mean ratios for each comparison. Both raw (Raw p) and

Tukey-adjusted (Adj p) p-values are provided. Comparisons that are significant at the 0.05 level are highlighted in pink. A summary of Dr. Hess' statistical analysis is included in **Appendix II.**

2.3 : EVALUATING MEMBRANE FLUIDITY BY USING CONFOCAL MICROSCOPY

We evaluated membrane lipid order to determine whether the lipid composition of the granulosa cell membrane changed with aging. One possible cause of reduced fertility is a change in these highly ordered lipid compartments, particularly in insulin-resistant animals. The rapid development of fluorescent dyes during the last decade had increased the use of fluorescence microscopy techniques for the study of biological systems. Di-4-ANEPPDHQ is an environmentally sensitive lipid probe which differentiates the liquid-ordered phase from lipid-disordered phase. It has a useful signal-to-noise ratio and is not readily internalized (94). Obaid and coworkers (82) have shown that the probe is sensitive to differences in lipid packing within membrane micro domains where local cholesterol concentrations are high and lipid packing affects the dye's emission spectrum.

Changes in membrane lipid order appear as a spectral blue shift in fluorescence from ordered to disordered phases. These images typically take the form of a normalized intensity ratio image (83). In these studies, Di-4-ANPPDHQ was excited by a 488 nm source and underwent a spectral shift in fluorescence emission to lower wavelengths in the presence of cholesterol and/or more ordered lipid environments. In disordered lipid environments, the probe emits with emission maxima at 628 nm and shifts to an emission maxima of 588 nm emission in cholesterol-enriched lipid environments (95).

2.3.1: Cell treatments

Equine granulosa cells were aspirated from dominant follicles and after removing cell debris, were incubated overnight in medium containing -FBS/-30 mM glucose or +FBS/+ 30 mM glucose. Cell culture media was then replaced before treating cells with human 1 μM EGF, 10 nM IGF-1, 100 nM progesterone, 100 nM insulin, or 100 nM hCG for 1 hour.

2.3.2: Staining cells with Di-4-ANPPDHQ

To evaluate lipid packing and lipid domain structure in equine granulosa cells, we labeled granulosa cells with 4 μM Di-4-ANPPDHQ in a dark room at room temperature, incubated cells for 30-60 min, and then washed cells 3 times with phosphate-buffered saline pH 7.4 before imaging fluorescence emission using an Olympus Fluoview IX-71 confocal microscope system. 488 nm exciation was used together with dual channel emission detection using a 630 nm dichroic beam splitter and a 510 nm and 570 nm long pass filter. A red/green channel spectral merge was used to evaluate differing degrees of lipid order (**Figure 2.2**). Initial experiments evaluated whether overall lipid order was reduced in granulosa cells from older mares compared to younger mares. In some experiments, effects of hormone binding on membrane lipid order was then evaluated. In these experiments, cells were incubated with hormone for 1 hour prior to labeling with Di-4-ANPPDHQ.

2.3.3: Data analysis

Background correction, fluorescence intensity ratio calculation and red/green overlay creation was performed by using the Image J program (rsbweb.nih.gov/ij). Increases in the fluorescence emission ratio indicate an increase in plasma lipid disorder.

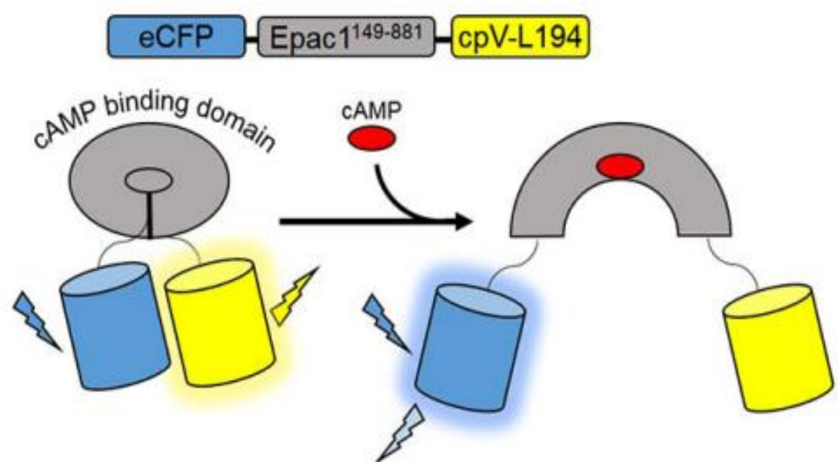


Figure 2.1: ICUE3 consists of an Epac1 149−881 sensing unit flanked by an ECFP donor and a cpV-L194 acceptor-reporting unit. Upon binding cAMP, the sensor switches from a high FRET to a low FRET conformation (from (96)).

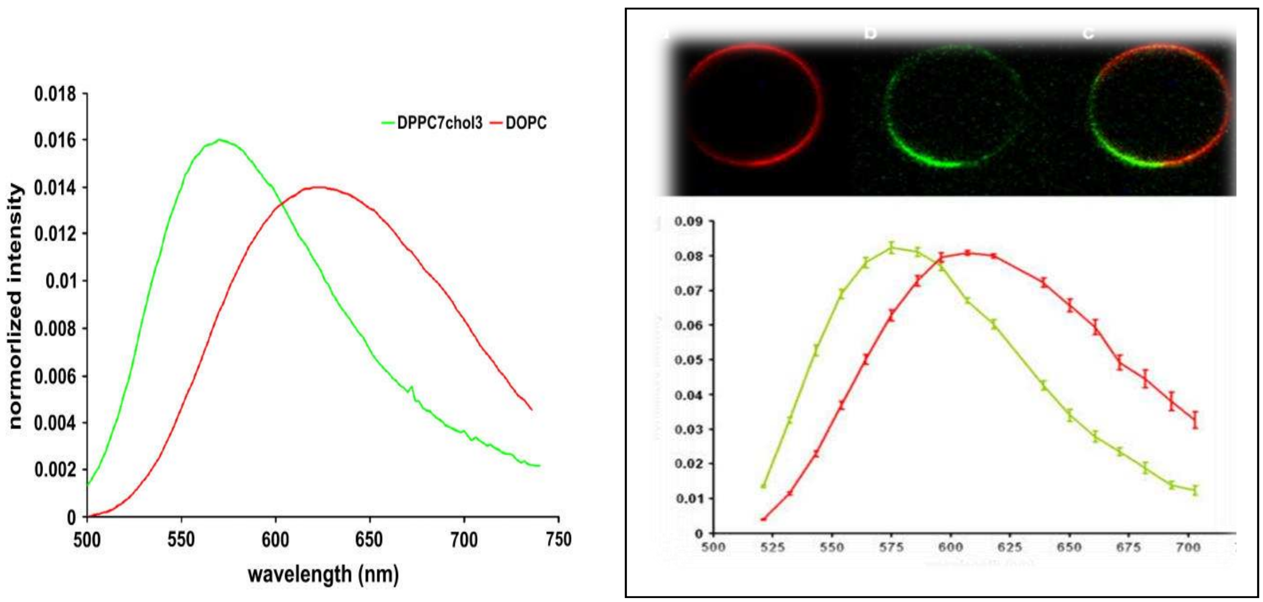


Figure 2.2: This figure shows the shift in Di-4-ANPPDHQ emission when it associates will more ordered membrane regions (green) and less-ordered regions (red) (from (83)). Samples were illuminated with 488 nm light.

CHAPTER 3. RESULTS

3.1: GENE EXPRESSION BY GRANULOSA CELLS FROM YOUNG OR OLDER MARES

3.1.1: Comparison of the effects of medium containing -FBS/-glucose and medium containing +FBS/+glucose on ΔCt values for IR, IGF1R and GLUT4

Our initial qRT-PCR studies compared the effects of medium containing no additional FBS or glucose or medium containing FBS and 30mM glucose on ΔCt values for *IR, IGF-1R* and *GLUT4*. **Figure 3.1.1** shows a significant difference in ΔCt values for *IGF-1R* expression between young and old animals for cells incubated in medium containing +FBS/+30mM glucose ($p<0.06$). Older animals had a ΔCt value of 12.42 ± 1.44 compared to younger animals where ΔCt was 9.81 ± 0.81 indicating higher expression of *IGF-IR* in younger animals in medium containing FBS and high levels of glucose.

3.1.2: Effects of age on gene expression by granulosa cells from young and older mares

The strongest effects of age were seen in the expression of *AMPK* subunits. **Table 3.1.1** summarizes the significant differences in gene expression for granulosa cells obtained from young and older animals. Results for specific genes are discussed below. There were significant differences in ΔCt for expression of *AMPKβ1* between young and old mares when cells were incubated in the absence of FBS and 30mM glucose ($p<0.005$). Older animals consistently demonstrated higher expression of *AMPKβ1* in untreated granulosa cells ($p<0.001$) and in cells treated with insulin ($p<0.003$), EGF ($p<0.05$), IGF-1 ($p<0.04$) or progesterone ($p<0.04$) in the absence of FBS and 30mM glucose. ΔCt values for *AMPKβ1* expression in +FBS/+glucose medium demonstrated a significant difference between young and old mares only following EGF treatment ($p<0.006$) with older animals showing higher expression. There was a significant difference between expression of *AMPKα2* in the absence of FBS and 30mM glucose between otherwise untreated granulosa cells from young and old mares ($p<0.01$) with higher expression of *AMPKα2* in older animals. *AMPKγ3* expression was significantly increased in granulosa cells from older animals compared to younger animals following incubation in -FBS/-glucose medium in untreated granulosa cells ($p<0.02$) and in cells treated with insulin ($p<0.03$). In presence of FBS and 30 mM glucose, cells from older animals treated with hCG had a higher expression of *AMPKγ3* ($p<0.007$) than did younger animals. There was also a significant increase in *AMPKβ1* expression in older animals following hCG treatment in absence of FBS and 30 mM glucose ($p<0.06$).

Table 3.1.1. also summarizes a significant difference in *GLUT4* expression in granulosa cells incubated with +FBS/+glucose and treated with hCG ($p<0.05$). There was >15-fold higher expression of *GLUT4* in older animals in response to hCG, the largest effect of age seen in these studies. *GLUT4* gene expression in young and old mares as well as hormone effects on *GLUT4* expression are shown in **Figure 3.1.2**. As discussed above, *IGF-1R* expression was also higher in otherwise untreated cells from older animals pre-incubated in +FBS/+glucose medium (**Table 3.1.1**).

3.1.3: Treatment effects on granulosa cells from either young or older mares

Table 3.1.2. summarizes significant differences in gene expression in granulosa cells from young and old mares incubated in either -FBS/-30mM glucose or +FBS/+30 mM glucose

and for untreated cells or cells treated with 100 nM insulin, 10 nM IGF-1, 100 nM hCG, 100 nM progesterone or 1 μM EGF. For animals within the same age group, most significance effects were shown for otherwise untreated cells from older animals preincubated in -FBS/-glucose versus +FBS/+glucose or for insulin-treated cells from older animals preincubated in -FBS/-glucose versus +FBS/+glucose. There were also significant differences in *IR* expression ($p<0.05$) in the absence of -FBS/-glucose following insulin treatment and treatment when compared with either hCG ($p<0.02$), EGF ($p<0.006$), IGF-1, ($p<0.02$), or progesterone treatments ($p<0.03$) in old age (**Figure 3.1.3**). When cells were incubated in +FBS/+30 mM glucose-containing medium, the only significant difference occurred between insulin and IGF-1 treatments ($p<0.05$).

3.2: Intracellular cAMP levels in granulosa cells from young and older animals

We examined hormone responsiveness of granulosa cells from young and old mares fed a normal hay-based diet to evaluate whether there was reduced hormone responsiveness with aging. Granulosa cells from young and older mares with isolated and then incubated overnight in medium containing ICUE3, a cAMP reporter molecule, and containing either +FBS/+30 mM glucose or no FBS or additional glucose. After an overnight incubation, ICUE3 was imaged briefly before treatment of cells with either 100 nM insulin, 1 μM EGF, or 10 nM IGF-1. Changes in cAMP levels within a single cell as a result of 1 hr hormone treatment were monitored as changes in intramolecular FRET values for the ICUE3 reporter. Decreases in ICUE3 intramolecular FRET with hormone treatment indicated an increase in intracellular cAMP. These data are shown in **Table 3.2.1**.

3.2.1: Effect of animal age on hormone signaling by granulosa cells:

There was a significant difference between young and old mares fed a normal diet when granulosa cells were pre-incubated in media with +FBS/+30 mM glucose and treated with 10 nM IGF-1. Intracellular cAMP levels were increased in old mares compared to younger mares ($p<0.01$) with ratios of 1.150 and 1.066, respectively. This effect of age is indicated in **Table 3.2.1** by an upper-case letter A in the right column of statistically different IGF-1 responses for young and older mares. **Figure 3.2.1** provided by Dr. Ann Hess in the Department of Statistics shows the variability in cell responses to specific hormones and shows clearly the difference between IGF-1 response in cells obtained from young and older animals.

3.2.2: Effects of hormone treatment

As shown in **Table 3.2.1**, most of the statistically significant differences between treatments were seen in cells from older animals and reflected differences in IGF-1 response in presences of +FBS/+glucose and responses to other hormones either in the presence or absence of FBS and additional glucose. This was the case for insulin-treated cells, EGF-treated cells and IGF-1 treated cells. Differences in hormone responsiveness are indicated by a lower-case letter in the right column of **Table 3.2.1**. There were also significant differences between treatments for cells from either young or older animals pre-incubated in either -FBS/-glucose or +FBS/+glucose (**Table 3.2.1**).

3.3: Single cell assays of intracellular cAMP in granulosa cells from young and older mares fed a proprietary diet high in anti-oxidants

In a subsequent study, we examined granulosa cells from mares fed a normal diet and mares fed an anti-oxidant enhanced proprietary diet. These mares were included in a larger study by Dr. Elaine Carnevale at Equine Reproduction Laboratory at Colorado State University. The goal of our project was to determine whether mares fed an enriched diet containing anti-oxidants exhibited improved responsiveness to insulin and epidermal growth hormone as measured by intracellular cAMP levels. As with the first study, granulosa cells obtained from mares in this study were pre-incubated overnight with medium containing the vector for ICUE3, a cAMP reporter molecule, and either -FBS/-30 mM glucose or +/FBS/+30 mM glucose. ICUE3 expressed in granulosa cells was then imaged briefly before treating cells with 100 nM insulin, 1 μM EGF, or 10 nM IGF-1.

3.3.1: Effects of an anti-oxidant diet compared to a normal diet

There was improvement in hormone responsiveness seen in granulosa cells from animals fed a diet rich in anti-oxidants when compared to age-matched animals fed a normal diet. This was seen in older mares following insulin ($p<0.0002$) and IGF-1 treatment ($p<0.01$) and in cells from diet-fed younger mares pre-incubated in +FBS/+30 mM glucose medium and treated with EGF treatment ($p< 0.02$). These results are summarized in **Figure 3.3.1.** Dr. Ann Hess provided the statistical analysis of the data.

Interestingly, there were also significant difference in older animals compared to younger animals when both groups were fed an enriched diet and cells were treated with insulin ($p<0.01$) or EGF ($p<0.05$). This suggested older animals experienced a greater benefit of diet than did younger animals when granulosa cells were exposed to a high glucose environment. **Figure 3.3.1,** was provided by Dr. Ann Hess. **Table 3.3.1** summarizes these data which are also contained, together with Dr. Hess' statistical analyses, in **Appendix II.**

3.4: Membrane lipid order in granulosa cells from young and older mares

Initial experiments evaluated whether overall lipid order was affected in granulosa cells from a young mare compared to a old mare when cells were pre-incubated in medium containing either FBS alone or high glucose alone and then treated with 100 nM insulin for 1 hour. The results shown in **Figure 3.4.1.** These studies used a lipid probe, Di-4-ANPPDHQ, which emits with emission maxima at 628 nm in more disordered lipid environments and shifts to an emission maxima of 588 nm emission in cholesterol-enriched lipid regions.

As shown in **Figure 3.4.1**, values for the ratio of fluorescence intensity at 630/588 nm indicates that cells from the older animal have more ordered membranes than cells from younger animals for all the media formulations used to pre-incubate granulosa cells and both in the presence and absence of insulin. Because there were no significant differences between cells preincubated with FBS alone or glucose alone and cells pre-incubated with both FBS and additional glucose, our subsequent experiments evaluated lipid order following preincubation of cells in medium that did not contain FBS or additional glucose or in medium that contained FBS and 30mM glucose.

We then examined the effects of hormone binding on membrane lipid order. These data are summarized in **Table 3.4.1**. In these experiments, cell culture media was replaced by serum-free media or with medium containing FBS and 30 mM glucose for 24 hours before treating cells for 1 hour with 1 μM EGF (Sigma;), 10 nM IGF-1, 100 nM progesterone, 100 nM insulin (Sigma) or 100 nM hCG (Fitzgerald). In these experiments, there were significant differences between membrane order in cells from young and old mares following EGF, hCG, and IGF-1 treatments when granulosa cells were pre-incubated in -FBS/-glucose medium. In general, older animals had more ordered membranes than did younger animals under conditions where there were significant differences (**Figure 3.4.2).** Similarly, there were significant differences between membrane lipid order for young and old mares in otherwise untreated granulosa cells and following EGF, hCG, and IGF-1 treatments when granulosa cells were preincubated in medium containing FBS and 30mM glucose (**Figure 3.4.3**). Again, older mares had more ordered membranes than did young mares under conditions where there were significant effects of age.

There were also significant effects of hormone treatments for cells from young (**Figure 3.4.4**) or older mares (**Figure 3.4.5**) when comparing cells that were pre-incubated in -FBS/-30 mM glucose or +FBS/+30 mM glucose. In younger animals, untreated or insulin-treated granulosa cells pre-incubated in +FBS/+30 mM glucose had less ordered membranes than did cells pre-incubated in medium without FBS or additional glucose (**Figure 3.4.4**). For older animals, there were highly significant differences between granulosa cells incubated in -FBS/-30 mM glucose compared to +FBS/+30 mM glucose medium for all treatments with more ordered membranes observed following pre-incubation in -FBS/-glucose (**Figure 3.4.5**).

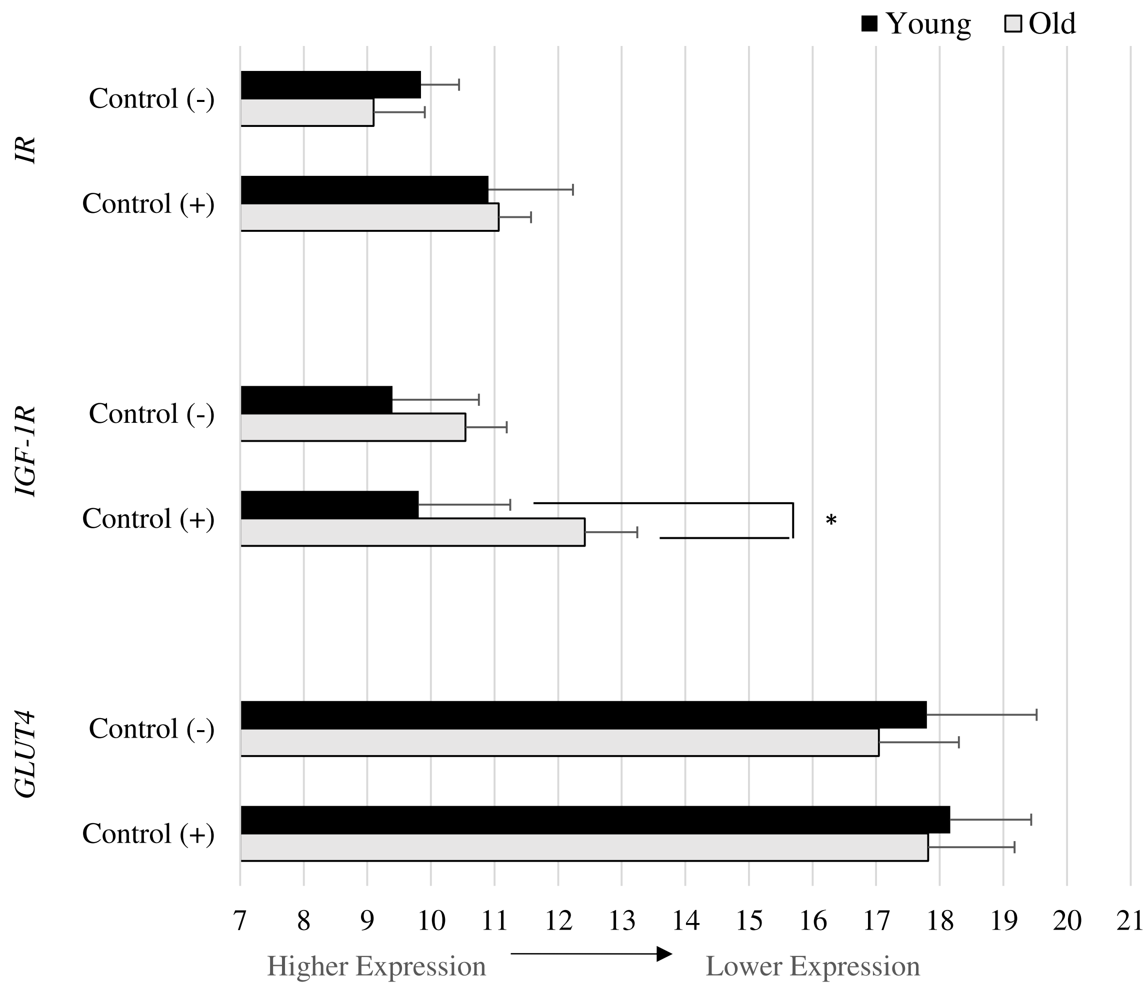


Figure 3.1.1: ΔCt values for *IR, IGF-1R and GLUT4* expression in granulosa cells from young and old animals incubated in medium containing -FBS/-glucose (-) or medium containing +/FBS/+glucose (+). Only *IGF-1R* expression in the presence of +FBS/+glucose showed a significant difference between young and old mares (p<0.06) (values indicated by *, p< 0.04).

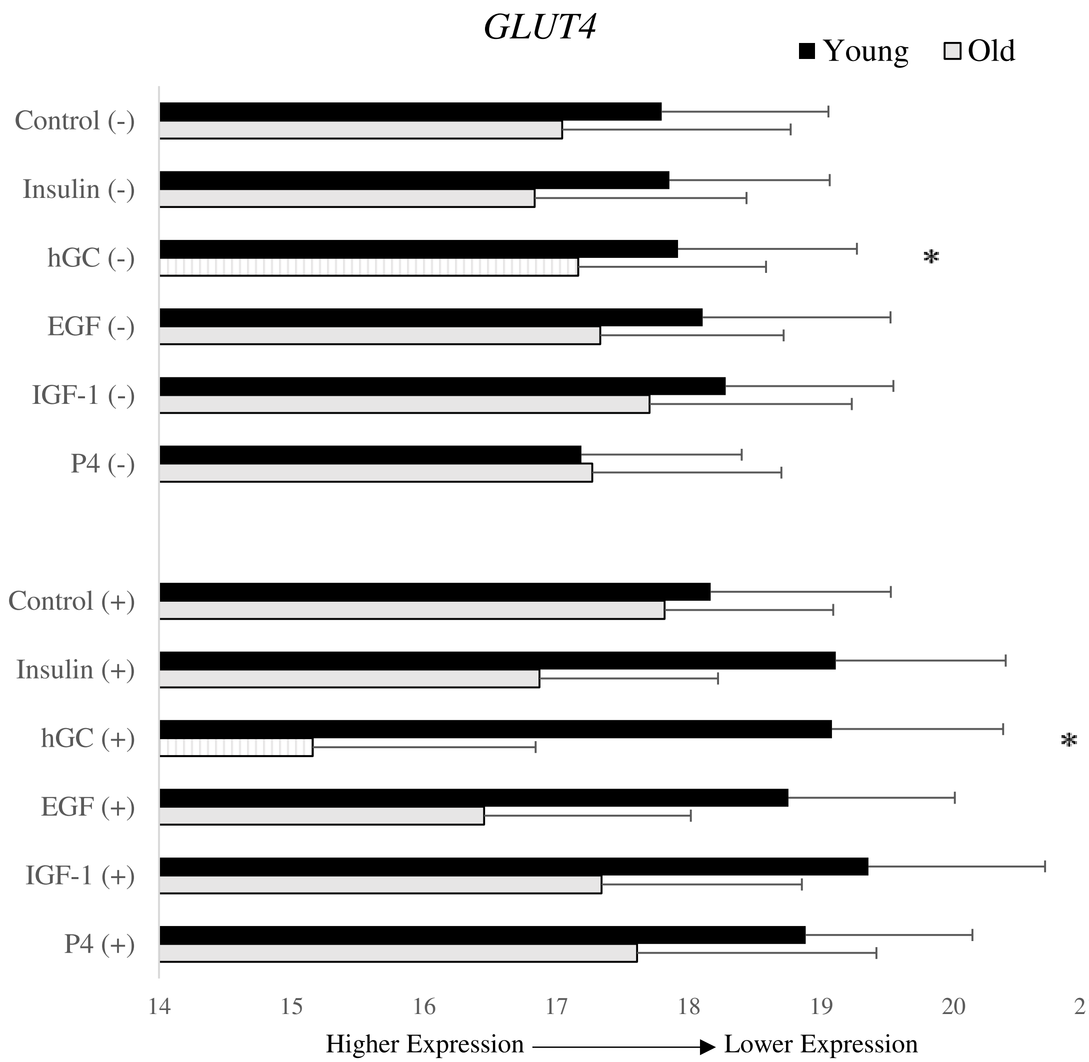


Figure 3.1.2: *GLUT4* expression in granulosa cells from younger and older mares. There was a significant difference between Glut4 expression from younger and older animals when cells were pre-incubated in +FBS/+30 mM glucose and treated with hCG (p< 0.05). There was also a significant difference between effects of preincubation medium -FBS/-glucose, and +FBS/+glucose on *GLUT4* expression in old age when cells were treated with hCG (values indicated by *, p< 0.04).

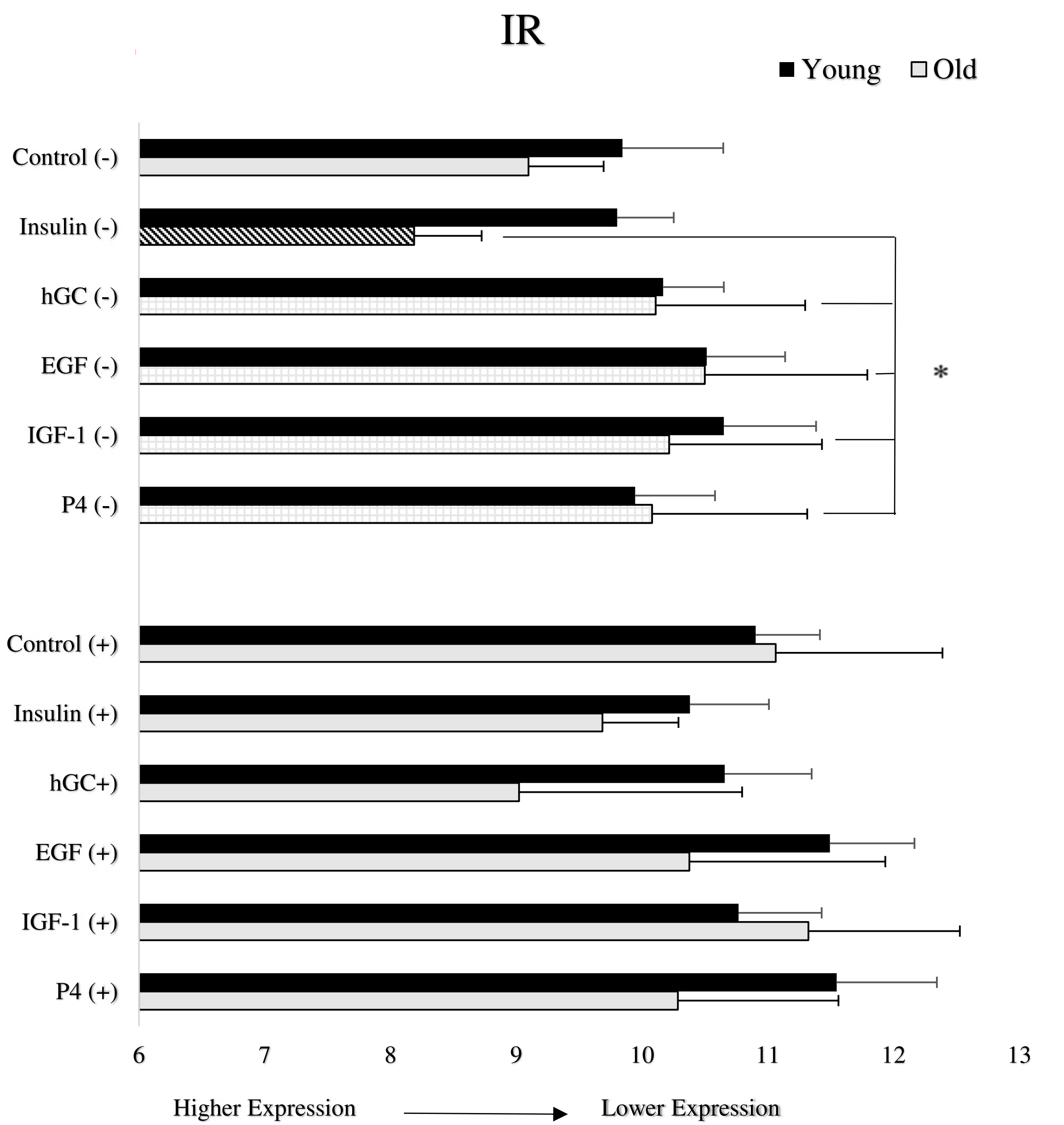


Figure 3.1.3: Expression of *IR* in granulosa cells from young and older mares in medium containing -FBS/-glucose or +FBS/+glucose. As indicated by an asterisk to the right of the bar graphs, there were significant differences between treatments for cells incubated in -FBS/-glucose and treated with insulin as compared to treatment with either hCG ($p<0.02$), EGF ($p<0.006$), IGF-1 ($p<0.02$) or progesterone ($p<0.03$). Where there were significant differences in hormone treatment, expression of *IR* was higher in response to insulin as compared to the other hormone effects.

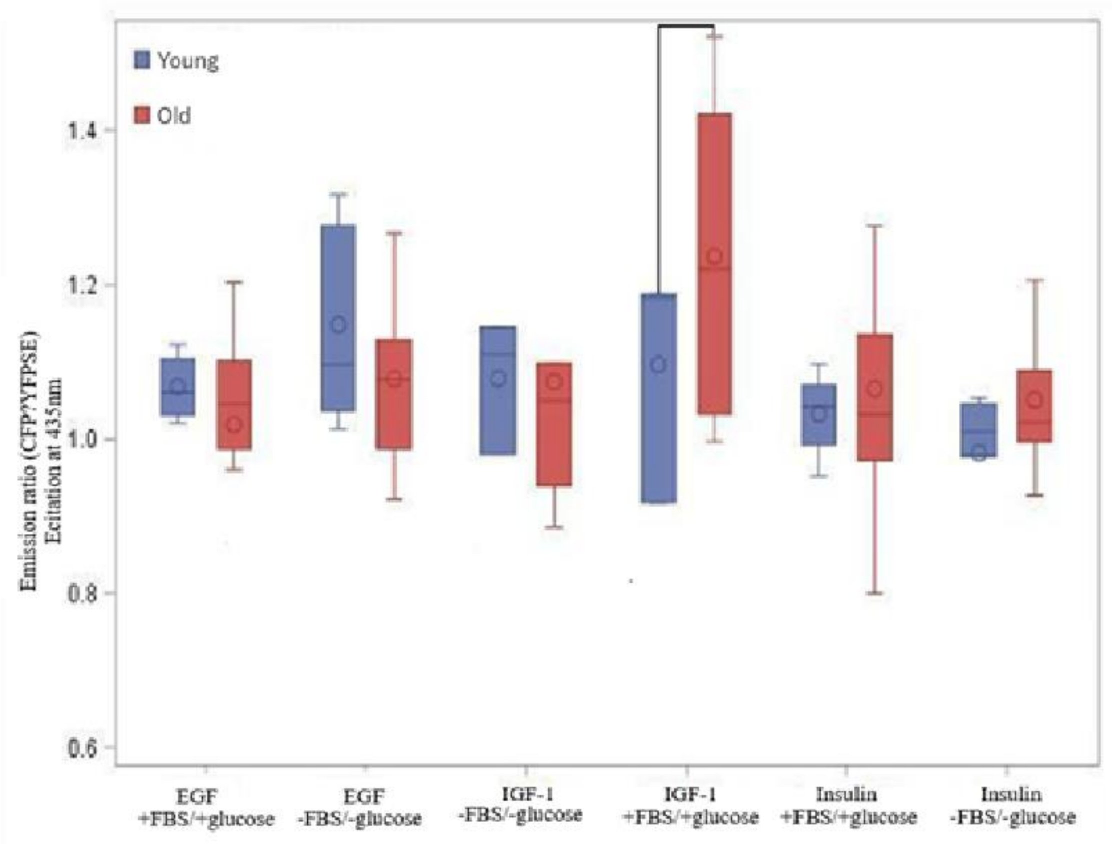


Figure 3.2.1: cAMP levels in granulosa cells from young and older mares pre-incubated in the absence (-) or presence of FBS and 30 mM glucose (+) and fed a normal diet. In IGF-1 treated cells, intracellular cAMP increased in older animals compared to younger animals ($p<0.01$). This figure was obtained from Dr. Ann Hess.

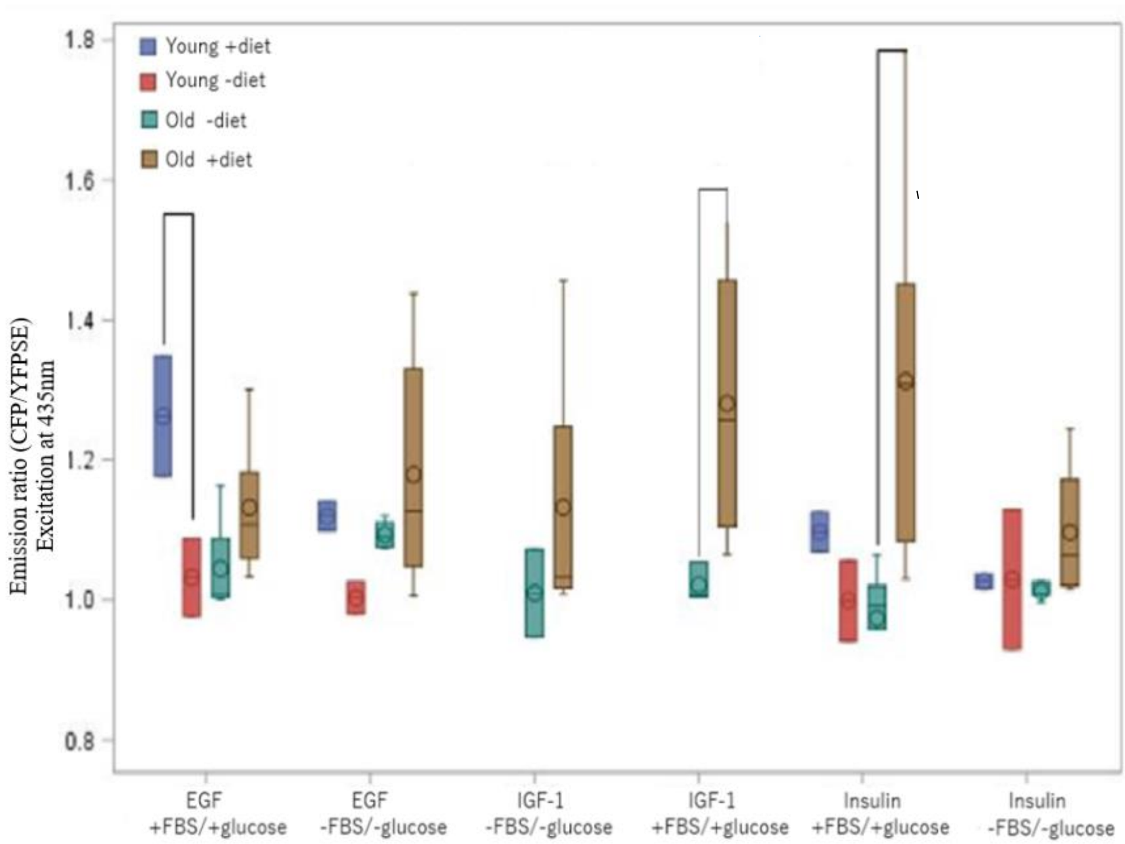
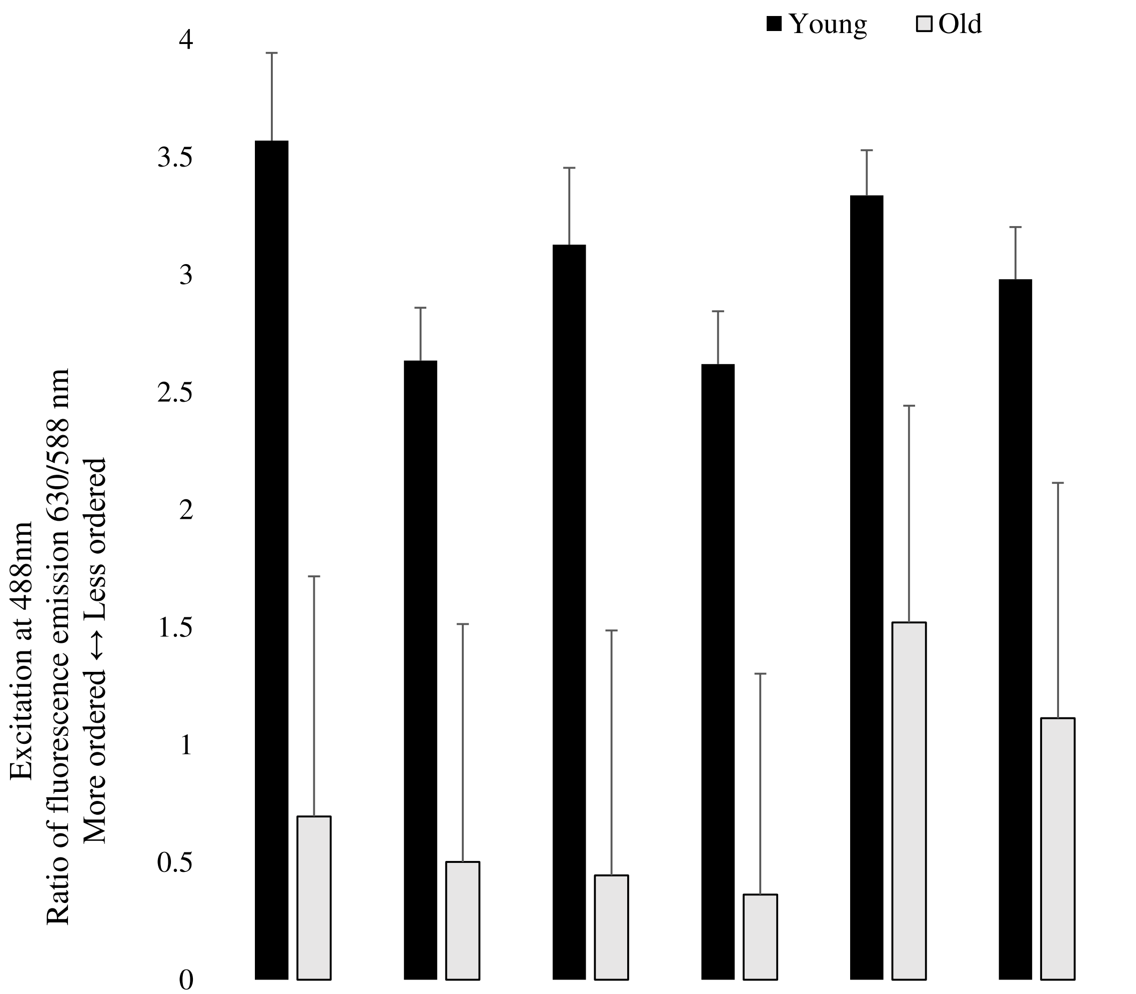


Figure 3.3.1: Effects of a normal diet and a proprietary diet enriched with anti-oxidants on intracellular cAMP levels pre-incubated in absence (-) or presence of FBS and 30 mM glucose (+). cAMP levels for EGF-treated cells in medium containing +FBS/+glucose (+) differed for young mares fed an enriched diet compared with young animals fed a normal diet. Also, there was different in old mares fed an enriched diet compared with old animals fed a normal diet for insulin and IGF-1 response. This figure was obtained from Dr. Ann Hess.



(-/+ insulin)	(-)	(+)	(-)	(+)	(-)	(+)
(-/+ FBS)	(-)	(-)	(+)	(+)	(-)	(-)
(-/+ glucose)	(-)	(-)	(-)	(-)	(+)	(+)

Figure 3.4.1: Comparison of the the relative "order" of membrane lipids from granulosa cells obtained from a younger animal (n=2) (black bars) and from an older animal (n=2) (white bars). Cells were pre-incubated in media containing either +/-fetal bovine serum, or 30mM glucose, +/-insulin or in medium containing +/-glucose and +/- insulin. These results indicate that under all conditions, the older animal had more ordered membrane lipids.

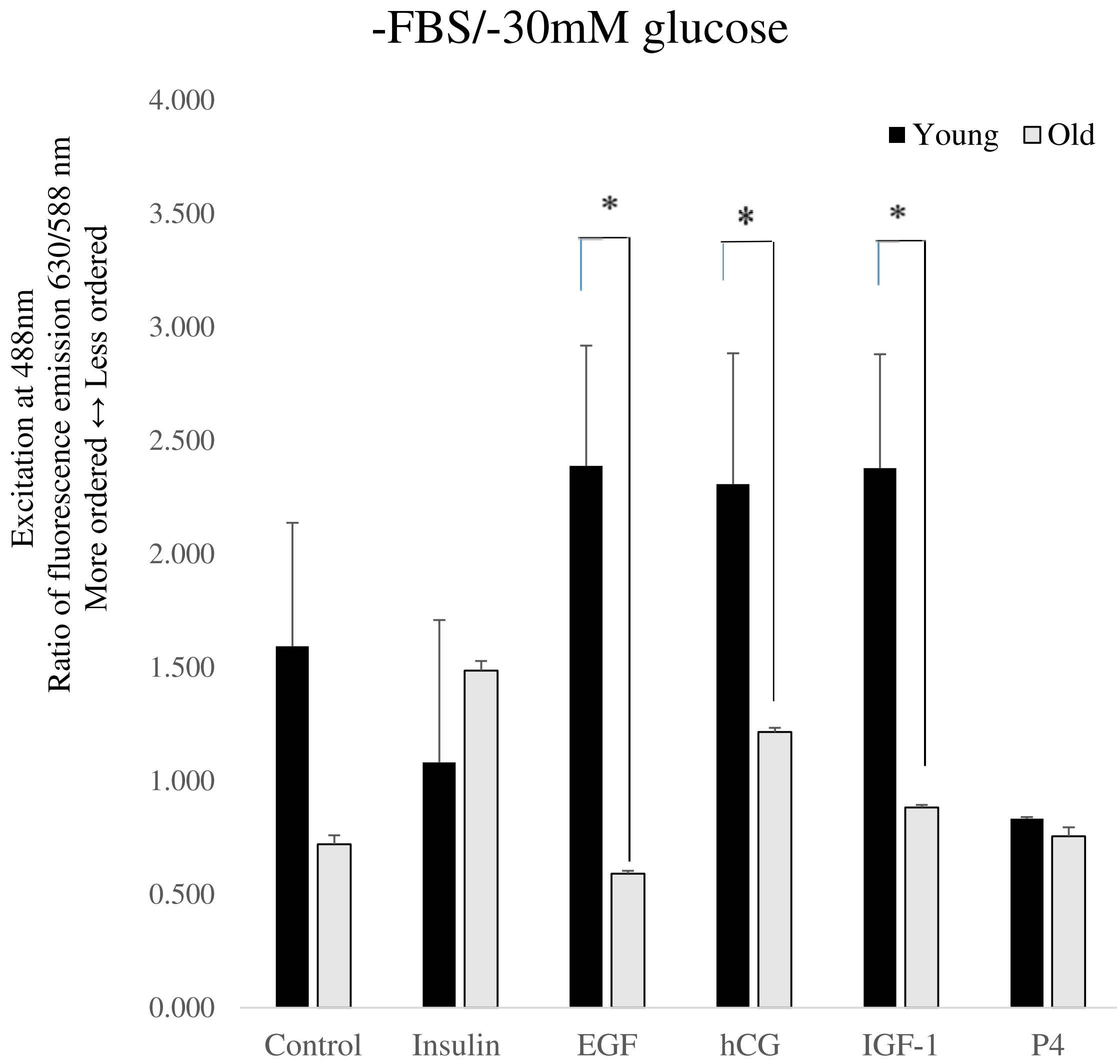


Figure 3.4.2: Effect of insulin, EGF, hCG, IGF-1 or progesterone on lipid order in young and older mares following pre-incubation of granulosa cells in -FBS/-30 mM glucose medium. As indicated by asterisks above the bar graphs, there were significant differences between young (n=10) (black bar) and old mares (n=8) (white bar) following treatment with EGF, hCG, or IGF-1 with younger animals exhibiting less ordered membranes.

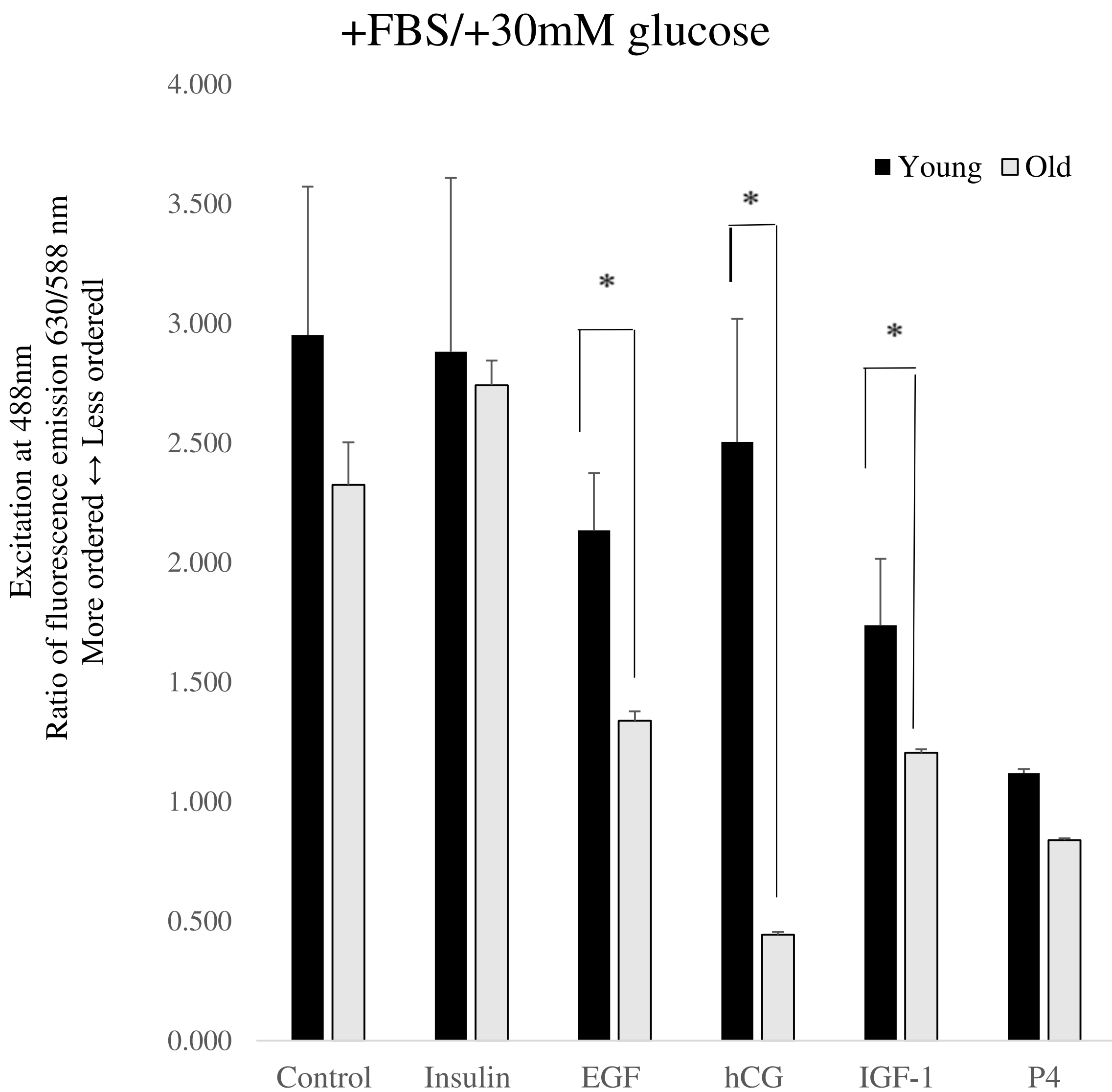


Figure 3.4.3: Hormone treatment of cells from young and older mares pre-incubated in medium containing +FBS/+glucose. As indicated by asterisks above the bar graphs, there were significant differences between young (n=10) (black bar) and old mares (n=8) (white bar) in untreated granulosa cells, EGF, hCG, and IGF-1 treatments with cells from older mares exhibiting more ordered membrane than cells from younger mares.

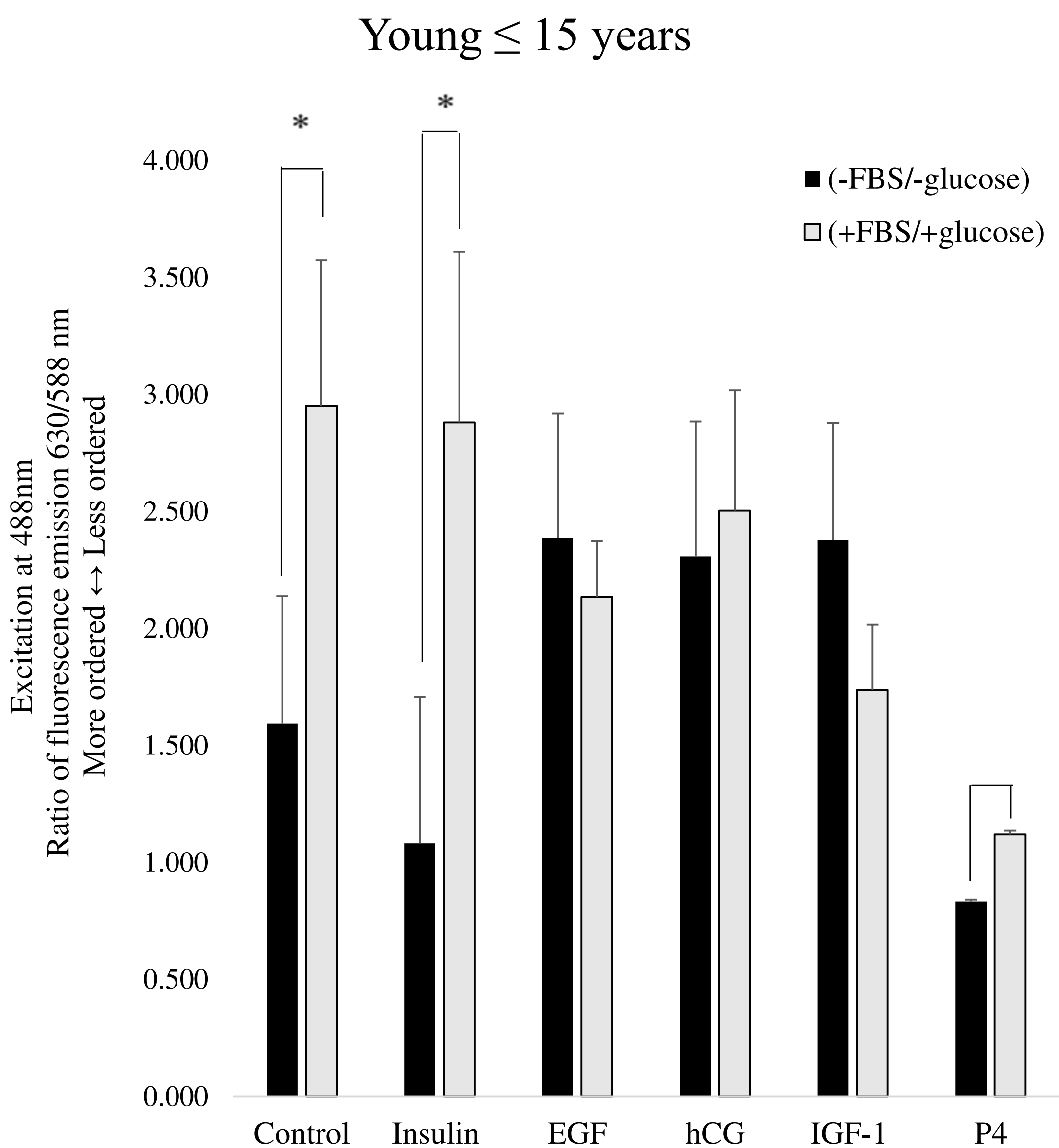


Figure 3.4.4: Effect of insulin, EGF, hCG, IGF-1 or progesterone on lipid order in young mares following pre-incubation of granulosa cells in -FBS/-glucose or + FBS/+glucose medium. As indicated by asterisks above the bar graphs, granulosa cells incubated in +FBS/+ glucose medium have more ordered membranes when cells are untreated or treated with insulin than do cells incubated in medium containing no FBS or additional glucose.

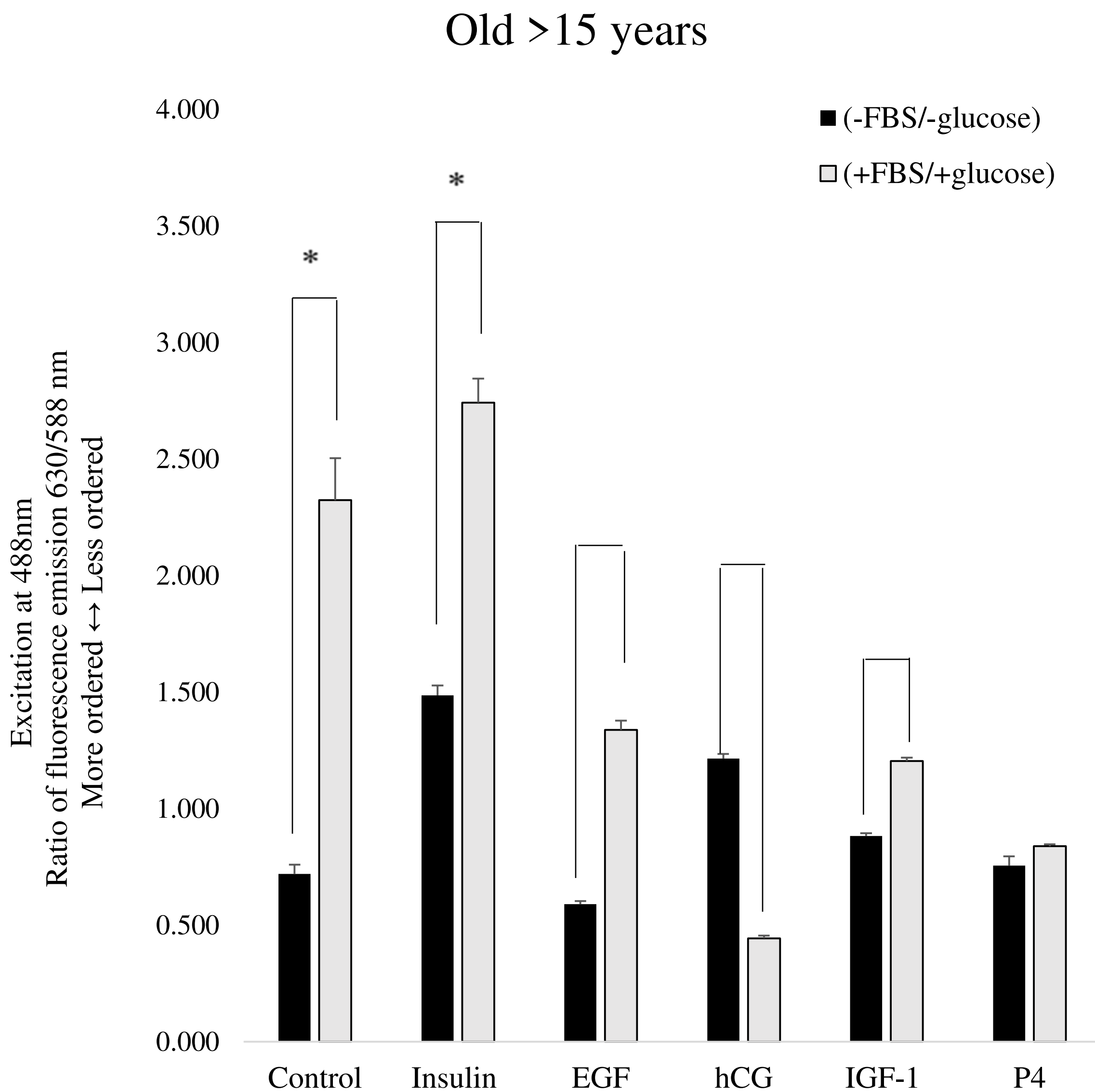


Figure 3.4.5: Effect of insulin, EGF, hCG, IGF-1 or progesterone on lipid order in older mares following pre-incubation of granulosa cells in -FBS/-glucose or + FBS/+glucose medium. As indicated by asterisks above the bar graphs, there were highly significant differences in membrane lipid order as a result of the medium used to pre-incubate cells. In older animals, +FBS/+ glucose medium generally resulted in less ordered membranes.

Table 3.1.1: Significant differences with age in expression of *AMPK* subunit genes, *GLUT4 and IGF-1R*. The specific gene is shown in the left column. Treatments that produced significant differences between young and old animals together with values for the fold change in ΔCt and p values are shown in columns on the right.

Gene	**Enhanced Medium (+FBS/ +glucose)**	**Treatment**	**Age**		**Fold-change**	
			Young ΔCt ± SE	Old ΔCt ± SE	Fold-change ($2^{-\Delta\Delta Ct}$)	p
AMPK α1	-	None	8.8 ± 0.8	6.8 ± 0.5	3.9	< 0.04
AMPK α2	-	None	10.1 ± 0.8	7.8 ± 0.5	5.1	< 0.01
AMPK α2	+	EGF	10.4 ± 0.7	8.5 ± 0.4	5.5	< 0.04
AMPK β1	-	None	11.1 ± 0.7	7.9 ± 0.7	8.6	< 0.001
AMPK β1	+	None	10.6 ± 0.8	9.1 ± 0.7	2.9	< 0.08
AMPK β1	-	Insulin	10.6 ± 0.6	7.8 ± 0.8	6.9	< 0.003
AMPK β1	-	hCG	9.9 ± 0.6	8.3 ± 0.7	2.5	< 0.06
AMPK β1	-	EGF	10.3 ± 0.5	8.6 ± 0.7	3.3	< 0.05
AMPK β1	+	EGF	11.0 ± 0.9	8.4 ± 0.7	5.9	< 0.005
AMPK β1	-	IGF-1	10.7 ± 0.4	8.8 ± 0.7	3.8	< 0.04
AMPK β1	-	P4	9.9 ± 0.4	8.6 ± 0.7	2.6	< 0.04
AMPK β1	+	P4	10.8 ± 0.4	9.1 ± 0.8	3.3	< 0.05
AMPK γ3	-	None	14.2 ± 0.6	11.3 ± 0.8	7.4	< 0.02
AMPK γ3	-	Insulin	12.9 ± 0.7	10.2 ± 1.1	6.3	< 0.03
AMPK γ3	+	hCG	13.4 ± 1.1	10.1 ± 0.6	10.4	< 0.007
IGF-1R	+	None	9.8 ± 0.8	12.4 ± 1.4	0.2	< 0.10
GLUT4	+	hCG	19.1 ± 1.3	15.2 ± 1.7	15.2	< 0.05

Table 3.1.2: Significant differences in gene expression in granulosa cells from either young or older mares incubated in either -FBS/glucose or +FBS/glucose. The gene is shown in the left column. Values for ΔCT for a specific treatment and age and values for $2^{-\Delta\Delta Ct}$ for expression of that gene and p values are shown in the columns to the right.

Gene	Treatment	Young Age		Old Age		Fold-change	
		Young -FBS/glucose ΔCt ± SE	Young +FBS/glucose ΔCt ± SE	Old -FBS/glucose ΔCt ± SE	Old +FBS/glucose ΔCt ± SE	Fold-change ($2^{-\Delta\Delta Ct}$)	p
IR	None	-	-	9.1 ± 0.6	11.1 ± 1.3	3.9	< 0.02
IR	Insulin	***-	-	8.2 ± 0.5	9.7 ± 0.4	2.8	< 0.07
AMPKα2	None	-	-	7.8 ± 0.5	9.2 ± 0.5	2.7	< 0.04
AMPKα2	Insulin	-	-	8.5 ± 0.6	9.8 ± 0.6	2.6	< 0.05
AMPKα2	hCG	9.0 ± 0.7	10.3 ±0.8	-	-	2.4	< 0.05
AMPKβ1	None	-	-	7.9 ± 0.7	9.1 ± 0.7	2.3	< 0.07
AMPKβ1	Insulin	-	-	7.8 ± 0.8	9.2 ± 0.5	2.7	< 0.03
AMPKβ1	Insulin	10.6 ± 0.6	9.5 ± 0.6	-	-	0.5	< 0.07
AMPKγ3	Insulin	-	-	10.2 ± 1.1	11.8 ± 0.9	3.1	< 0.08
IGF-1R	None	-	-	9.8 ± 0.8	12.4 ± 1.4	2.6	< 0.08
IGF-1R	EGF	-	-	11.7 ± 1.7	9.7 ± 1.6	0.2	< 0.06
GLUT4	hCG	-	-	17.2 ± 1.1	15.2 ± 1.7	0.3	< 0.05
GLUT4	P4	17.2 ± 1.2	18.9 ± 1.3	-	-	3.2	< 0.06

Table 3.2.1: Hormone-induced changes in intracellular cAMP in granulosa cells between young and old mares pre-incubated in the presence or absence of FBS and additional glucose.

Age	Number	Treatments	Mean (before)	Mean (after)	Ratio*
Young	11	Insulin (+)	0.93	0.95	1.02
	11	Insulin (-)	0.93	0.91	0.99 [a]
	7	EGF (+)	0.97	1.02	1.05
	7	EGF (-)	1.00	1.11	1.11 [a]
	5	IGF-1 (+)	0.88	0.93	1.07 [A]
	5	IGF-1 (-)	0.95	1.00	1.05 [e]
Old	25	Insulin (+)	0.93	0.95	1.02 [d]
	25	Insulin (-)	0.93	0.96	1.04 [c]
	14	EGF (+)	1.08	1.09	1.02 [b]
	14	EGF (-)	1.01	1.07	1.06 [f]
	10	IGF-1 (+)	0.88	1.01	1.15 [A, b, c, d, e, f]
	10	IGF-1 (-)	0.99	1.03	1.04

*Values with the same letter were significantly different between treatment ($p<0.05$). Values with lower case letters indicate significant differences in response to various hormone treatments while the values with the capital letter "A" exhibited a significance difference between cells from young and older animals.

Table 3.3.1: Significances differences in cAMP levels in granulosa cells from young and old mares fed either a normal diet or a diet high in anti-oxidants. P values for treatments producing significant differences between young and old mares, between normal diet and a diet rich in antioxidants, and/or in the presence or absence of FBS and 30 mM glucose are shown in the right column.

Age	Diet	Medium FBS/glucose	Treatment	n	Before	After	Ratio After/before	Significance
Young	+	+	Insulin	2	0.91	0.99	1.10	b, C
	+	-	Insulin	2	0.93	0.96	1.03	a
	+	+	EGF	2	1.04	1.31	1.26	a, b, A, B
	+	-	EGF	2	1.01	1.13	1.12	
Young	-	+	Insulin	11	0.93	0.95	1.02	D
	-	-	Insulin	11	0.93	0.91	0.99	
	-	+	EGF	7	0.97	1.02	1.05	A
	-	-	EGF	7	1.00	1.11	1.11	
	-	+	IGF-1	5	0.88	0.93	1.07	G
	-	-	IGF-1	5	0.95	1.00	1.05	
Old	+	+	Insulin	8	0.81	1.04	1.31	c,C, D, E
	+	-	Insulin	8	0.85	0.94	1.10	
	+	+	EGF	6	0.89	1.01	1.13	c
	+	-	EGF	6	0.91	1.08	1.20	
	+	+	IGF-1	4	0.75	0.95	1.28	F, G
	+	-	IGF-1	4	0.89	0.98	1.13	
Old	-	+	Insulin	25	0.93	0.95	1.02	E
	-	-	Insulin	25	0.93	0.96	1.04	
	-	+	EGF	14	1.08	1.09	1.02	B
	-	-	EGF	14	1.01	1.07	1.06	
	-	+	IGF-1	10	0.88	1.01	1.15	F
	-	-	IGF-1	10	0.99	1.03	1.04	

Table 3.4.1: Membrane fluidity between young and old mares granulosa cells incubated presence or absence of FBS/30 mM glucose. An ↑ in the right column indicates the relative magnitude of the increase in membrane lipid order.

Treatment	**Medium**	**Ratio Young (5)**	**Ratio Old (3)**	**Change in lipid order with old age**
Control	(-)	1.6 ± 0.6	0.7 ± 0.1	↑
Control	(+)	3.0 ± 0.6	2.3 ± 0.2	↑
Insulin	(-)	1.1 ± 0.6	1.5 ± 0.1	~
Insulin	(+)	2.9 ± 0.7	2.7 ± 0.1	~
EGF	(-)	2.4 ± 0.5	0.6 ± 0.1	↑
EGF	(+)	2.1 ± 0.2	1.3 ± 0.1	↑
hCG	(-)	2.3 ± 0.6	1.2 ± 0.1	↑
hCG	(+)	2.5 ± 0.5	0.4 ± 0.1	↑
IGF-1	(-)	2.4 ± 0.5	0.9 ± 0.1	↑
IGF-1	(+)	1.7 ±0.3	1.2 ± 0.1	↑
Progesterone	(-)	0.8 ± 0.1	0.8 ± 0.1	~
Progesterone	(+)	1.1 ± 0.1	0.8 ± 0.1	↑

CHAPTER 4: DISCUSSION

There were significant effects of mares' age on gene expression in granolosa cells which are summarized in **Table 3.1.1**. Most of the effects we noted were on the expression of *AMPK* subunits although there were significant effects on *GLUT4* and *IGF-1R* expression as well. In general, increased expression of selected *AMPK* subunits was observed in older animals and, with one exception, was seen in cells pre-treated with medium that did not contain FBS or additional glucose. The *AMPK β1* subunit was expressed at higher levels in older animals whose cells were pre-treated without FBS and glucose. In addition to *AMPK* effects, there was a decrease in *IGF-1R* expression in older animals although this occurred only when cells were pre-treated with medium containing FBS and additional glucose. There was also decreased expression of *GLUT4* following hCG treatment and, again, this occurred only when cells were pre-treated with FBS and additional glucose.

Although the underlying cause of this increased expression of the *AMPK* subunits is not known from this study, various cellular stresses such as prolonged exercise or nutritional deprivation can lead to AMPK activation. Nutritional deprivation of granulosa cells, i.e. glucose deprivation, may be sufficient to drive the increase in AMPK subunit expression seen in these studies.

The various subunits of the AMPK enzyme have distinct functions. AMPKβ isoforms are necessary for proper activation of AMPK and its localization to membranes (97). Both AMPKα1 and AMPKα2 are activated in response to energy stress (98) and are both affected by exercise which creates additional energy demands on the organism. Interestingly however, *AMPKα1* expression increases after one month of exercise while AMPKα2 demonstrates no change (99).

AMPK is also activated in response to several pharmacological agents (100). AMPK regulates glucose uptake through effects on cell signalling intermediates which are involved in GLUT4 trafficking. This relationship between insulin-mediated signaling, GLUT4 trafficking and increased AMPK subunits may explain, in part, the observation that metformin used in the treatment of Type II diabetes affects AMPK activity (101) athough it remains controversial whether AMPK is absolutely required for the glucose lowering effects of metformin (102).

AMPKα2 expression in aging animals was also affected by EGF treatment in our studies. Pre-incubation of granulosa cells with FBS and 30mM glucose followed by EGF treatment increased the expression of *AMPKα2* and *AMPKβ1. AMPKβ1* expression also increased in older animals with EGF treatment when cells were preincubated without FBS and 30mM glucose.

EGF effects on granulosa cells were of interest because, despite a corresponding effect of EGF on lipid order in membranes from older animals, granulosa cells did not exhibit increased cAMP signaling in response to EGF. Modeling of EGF-mediated signalling by its receptor suggests receptor dependence on lipid environment (103) and a complex relationship with membrane rafts that may involve coallesing of smaller raft domains (104). This coallesing of smaller rafts into larger structures may be sufficient to the increase in lipid-ordered domains that are seen in our studies.

Despite EGF effects on the formation of EGF receptor dimers (105) and larger receptor complexes (106), there is, in our hands, no increase in EGF signaling. Because we would normally anticipate an increase in EGF-dependent signaling in these experiments, it remains an open question as to whether there is actually no increase in EGF signaling under these conditions or, alternatively, whether ICUE3 is not sufficiently sensitive to detect increased EGF signaling.

Also of interest in these studies is whether insulin responsivness is affected by aging. We originally hypothesized that the ratio of expression of *IR* to *IGF1R* genes would affect the relative numbers of these receptors and be predictive of insulin and IGF-1 responsiveness in both younger and older animals. We assumed that assembly of IR homodimers, IGF-1R homodimers and IR·IGF-1R hybrid receptors is dependent on available IR and IGF-1R monomers and that a ratio of ΔCt for *IR* relative to ΔCt for *IGF-1R* of 1.0 could be expected to produce assembly of equal numbers of IR homodimers, IR·IGF-1R hybrid receptors and IGF-1R homodimers in the endoplasmic reticulum (107). When ratios of ΔCt for *IR* relative to ΔCt for *IGF-1R* are greater than 1.0, synthesis of IR monomers would be expected to be lower than synthesis of IGF-1R monomers and, as a result, there would be increased formation of IGF-1 receptor homodimers and IR·IGF-1R hybrid receptors compared to insulin receptor homodimer formation. Similarly, when ratios of ΔCt 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 will be a predominance of IR_2 homodimers assembled in the endoplasmic reticulum. If these assumptions pertain to receptor expression in granulosa cells, granulosa cells from older mares that have been pre-incubated in medium that does not contain FBS or 30 mM glucose would be predicted to express approximately equal numbers of insulin receptors, IGF-1 receptors and hybrid receptors based on levels of *IR* and *IGF-1R* gene expression (**Table 4.1**). When cells are pre-incubated in FBS and 30mM glucose and the expression of *IR* increases in older animals, we would predict that there is increased assembly of IR homodimers at the expense of IGF-1 receptor homo-dimers and hybrid receptors and insulin and IGF-1 responsiveness would be altered accordingly.

Although there was a marked increase in *IR* expression in granulosa cells from older animals when cells were pre-treated with FBS and additional glucose, there was no accompanying increase in insulin responsiveness as measured by an increase in intracellular cAMP. When granulosa cells were preincubated without FBS or additional glucose and equal numbers of insulin receptors, IGF-1 receptors and hybrid receptors were predicted, there was a small increase in cAMP signaling that was not significant. Thus predicted changes in the ratio of *IR*:*IGF1R* expression did not affect cell signaling by granulosa cells, at least on the timescale of these experiments.

Nevertheless, the relationship between gene expression and cell signaling is probably not as simple as proposed above for formation of IR and IGF-1 receptors. In particular, the membrane environment may also be important in cell signaling by a number of receptors including IR, IGF-1R and EGF receptors. Arguably the most pronounced effect of age in these studies occurred in studies of membrane lipid order. Generally, older animals had more ordered plasma membranes than did younger animals. This increase in lipid order in granulosa cell membranes may be due to a number of events that occur either in isolation or concurrently. Membrane lipid order is related to the lipid composition of the membrane including relative amounts of unsaturated phospholipids, the specific phosphospholipids in the inner and outer leaflets and membrane cholesterol content. As bilayer asymmetry is lost and membrane lipids such as phosphatidylserine are redistributed in the outer membrane leaflet, one might expect a corresponding decrease in plasma membrane order. Phospholipids with unsaturated acyl-chains form membranes in which membrane fluidity, a reflection of membrane lipid disorder, is high. In diabetes, there is a decrease in unsaturated free fatty acids and phospholipids (108-111). Decreased fluidity in the hydrophobic regions of diabetic erythrocyte membranes was likely due to a decrease in unsaturated phospholipids (108-111).

Changes in membrane fluidity with aging may also reflect membrane cholesterol content. Cholesterol concentration in the plasma membrane is generally expressed as the cholesterol-protein ratio. Membrane fluidity, as evaluated in studies using fluorescence polarization and diphenyl-hexatriene and 1-(4-trimethylamino)-6-phenylhexa-1, 3, 5-triene, decreased as the ratio of cholesterol-protein increased in membrane microdomains (112). Thus, in our studies, one factor that would be expected to alter membrane lipid order is the relative amount of cholesterol contained in granulosa cell membranes.

Interestingly, these studies also demonstrated that diet has a major effect on cell signaling. When older mares received a diet rich in anti-oxidants, hormone responsiveness improved (**Table 3.2.1**). The most striking effects were seen with insulin or IGF-1 treatments of granulosa cells from diet-fed old mares preincubated with FBS and additional glucose when compared to insulin or IGF-1 responsiveness of cells from older mares fed normal diet. Both hormones required a high glucose environment; we did not see comparable hormone responses in cells pre-incubated in medium that did not contain FBS or additional glucose.

We also compared hormone responsiveness in diet-fed younger mares with hormone responses in diet-fed older mares. We found that the effect of antioxidant supplementation was only clear in old mares when granulosa cells were treated with 100 nM insulin and that there was no effect on cells from younger animals under the same conditions. Thus, diet effects were inconsistent between the two age groups. The caveat here is that there were comparatively few diet-fed young mares and this may affect our results.

Nonetheless, these results suggest that antioxidants correct cell responses to insulin and IGF-1 in older animals where cell metabolism may be disrupted. The specific site or sites of action are not clear from this study but may include actions of the glucose transporter GLUT4, signaling pathways involved in insulin responsiveness or protection from metabolic disorders through increased insulin sensitivity (113). A similar study in diabetic animals and humans showed that short-term of supplementation decreased insulin levels in early disease (114-116).

In lipid fluidity, our results indicate that under all conditions, the older animals had more ordered membrane lipids than did young animals. Membrane fluidity is related to the lipid composition of membranes and can affect receptor ligand binding and cellular response (117). Previous investigators have shown an age-dependent increase in lipid order which was correlated with an increased in the molar ratio of cholesterol to phospholipid in the membranes isolated from aged animals. As an example, the effect of aging on the lipid composition and fluidity of rat peritoneal macrophage membranes has been studied in detail with older animals exhibiting an age-dependent increase in the molar ratio of cholesterol/phospholipid (118). Because the molar ratio of cholesterol to phospholipids is considered to be a main determinant of lipid fluidity of both artificial and biological membranes (119), our results in granulosa cells are consistent with observations in other cell types.

Together these data suggest that the two primary effects of aging on granulosa cells are an increase in AMPK activity, which is consistent with decreasing availability intracellularly of nutrients needed for cell function. The other effect of aging is on membrane lipid order, which is probably the result of an increase in the cholesterol-to-phospholipid ratio within cells. Importantly, preliminary studies suggest that an improved diet may reverse some of the effects of aging and permit older animals to function at a metabolic level more closely resembling younger animals.

Table 4.1: The ratio ΔCt (*IR*) to ΔCt (*IGF-1R*) for *individual* young mares less than 15 years old (n = ten animals) and older animals greater than 15 years old (n = eight animals). Shown are predicted effects of these ratios on expression of insulin receptor homodimers and IGF-1R homodimers. Note that, as ΔCt values *increase*, the amount of mRNA expressed *decreases*. Thus, ratios of ΔCt (*IR*) to ΔCt *(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 < 15 years		Older Mares > 15 years	
Treatment	ΔCt (IR) / ΔCt (IGF-1R)	interpretation	ΔCt (IR) / ΔCt (IGF-1R)	interpretation
Control (-)	1.16 ± 0.2 (10)	↑ IGF-1R	0.99 ± 0.2 (8)	IR ≈ IGF-1R
Control (+)	1.28 ± 0.2 (10)	↑ IGF-1R	0.89 ± 0.2 (8)	↑ IR
Insulin (-)	1.27 ± 0.3 (10)	↑ IGF-1R	1.22 ± 0.4 (8)	↑ IGF-1R
Insulin (+)	1.16 ± 0.2 (10)	↑ IGF-1R	1.29 ± 0.5 (8)	↑ IGF-1R
hCG (-)	1.31 ± 0.3 (10)	↑ IGF-1R	1.51 ± 0.7 (8)	↑ IGF-1R
hCG (+)	1.14 ± 0.2 (10)	↑ IGF-1R	1.34 ± 0.6 (8)	↑ IGF-1R
EGF (-)	1.29 ± 0.3 (10)	↑ IGF-1R	1.04 ± 0.2 (8)	IR ≈ IGF-1R
EGF (+)	1.35 ± 0.2 (10)	↑ IGF-1R	1.22 ± 0.3 (8)	↑ IGF-1R
IGF-1 (-)	1.13 ± 0.2 (10)	↑ IGF-1R	1.49 ± 0.2 (8)	↑ IGF-1R
IGF-1 (+)	1.25 ± 0.3 (10)	↑ IGF-1R	1.33 ± 0.2 (8)	↑ IGF-1R

CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

These studies demonstrate significant effects of aging in granulosa cells. There are changes in gene expression and effects on membrane lipid packing. An improved diet appears to improve insulin and IGF-1 responsiveness by granulosa cells. These studies, although revealing, require additional work in this area of interest. In particular, studies of gene expression in granulosa cells from animals fed an anti-oxidant enriched diet would be helpful. Although insulin responsiveness is improved in these animals, the effects of diet on a number of parameters including metabolic activity, assayed via expression of AMPK subunit expression, and expression of insulin receptor, IGF-1 receptor and GLUT4 is not known.

An additional benefit of these studies would be to increase the number of animals used for assays of cAMP. In particular, the sample size of young animals fed the anti-oxidant enriched diet was small. A major question of the diet study was whether older, but not younger, animals benefited from the improved diet both in terms of gene expression and hormone responsiveness. Additional experimentation using younger animals would help resolve this question. It is also important in cAMP studies to add experiments assessing the ability of cells to produce cAMP, which could easily be accomplished by adding control experiments testing responsiveness to forskolin.

In addition, because of the key role of glucose uptake in insulin resistance, these studies would benefit from assays of glucose uptake in individual granulosa cells. Glucose uptake is a key biological measurement for testing effects of activators or inhibitors in cell culture and the ability of cells to respond to insulin. Since a decrease in insulin responsiveness is a hallmark of metabolic disease in older animals, several strategies for examining a cell's ability to respond to insulin would be appropriate. One strategy would be to use radiolabeled glucose to measure cell responses to insulin. Alternatively, assessing insertion of the GLUT4 transporter in cell membranes using an ELISA-based assay for GLUT4 translocation would be useful (96). In particular, the assay of GLUT4 transporter insertion would address whether there is compromised signaling in granulosa cells that limits the utility of available GLUT4 transporter molecules.

Along these same lines, another assay of metabolic activity by granulosa cells would strengthen the conclusions we reached in these studies. Adam J. Chicco at Colorado State University has used mitochondrial assays to study differences in metabolic function in young and old animals (120). He showed that cardiolipin content in cardiac tissues was important for the activity of mitochondria involved in the oxidative generation ATP. These studies of mitochondrial function in granulosa cells would also be of interest because of the marked changes we observed in granulosa cell membranes in older animals, a change that could potentially affect mitochondrial membranes as well.

Other studies of interest in granulosa cells from aging animals would include a more detailed exploration of the relationship between expression of insulin receptor and IGF-1 receptor genes and receptor numbers in the cell membrane. What is clear in our studies is that granulosa cells from older mares express *IR* at amounts comparable to those seen in younger animals. It would be of interest to know whether there is some disruption in the relationship between expression of *IR* genes and actual numbers of receptors. This could be resolved through fluorescence correlation spectroscopy (FCS) and related photon counting histogram studies of membrane receptor numbers. If such measurements were combined with FCS and PCH studies of IGF-1 receptors, it would also be possible to determine whether the predicted numbers of receptor homodimers and heterodimeric receptors based on gene expression levels and described in Table 4.1 was reasonable.

In conclusion, the effects of animal aging on cell function represent an interesting and potentially important topic in human and equine medicine. Cells involved in reproductive function, because of their well-defined functions and well-characterized changes with age, are particularly suitable for such a study. Dietary changes, such as the use of anti-oxidants, can also be evaluated readily in terms of organ function. Moreover, results from such work are very likely relevant to aging in other systems and thus of more general interest.

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APPENDIX I: ΔCT RAW DATA FROM RT-PCR EXPERIMENTS

<u>KEY: GENES (T)</u>

T1; AMPKα1,

T2; AMPKα2,

T3; AMPKβ1,

T4; AMPKβ2,

T5; AMPKγ1,

T6; AMPKγ2,

T7; AMPKγ3,

T8; IGF-1,

T9; GLUT4

T10; IR

<u>KEY: Treatments (G)</u>

G0: Control

G1: Insulin

G2: human chorionic gonadotropin hCG

G3: EGF

G4: IGF-1

G5: Progesterone

<u>KEY: Media</u>

m: minus (without FBS and 30mM glucose)

p: plus (with FBS and 30mM glucose).

Trt: treatment

Gene	Glucose	AgeCat	Trt	n	mean	SD	SE
T1	m	Old	G0	8	6.835	1.273461	0.450237
T1	m	Old	G1	8	7.121563	1.137751	0.402256
T1	m	Old	G2	8	7.339688	1.474814	0.521426
T1	m	Old	G3	8	7.554688	1.479425	0.523056
T1	m	Old	G4	8	8.154375	1.811636	0.64051
T1	m	Old	G5	8	7.017813	1.84401	0.651956
T1	m	Young	G0	10	8.818	2.397768	0.758241
T1	m	Young	G1	10	8.76375	2.392102	0.756449
T1	m	Young	G2	10	8.4975	1.972325	0.623704
T1	m	Old	G3	10	9.022	2.348809	0.742759
T1	m	Young	G4	10	8.59975	2.12934	0.673356
T1	m	Young	G5	10	8.4865	1.874972	0.592918
T1	p		G0	8	7.534688	1.417745	0.501249
T1	p	Old	G1	8	7.600625	1.422009	0.502756
T1	p	Old	G2	8	7.8275	2.290763	0.809907
T1	p	Old	G3	8	7.6025	1.304677	0.461273
T1	p	Old	G4	8	8.539688	2.090178	0.738989
T1	p	Old	G5	8	7.865	1.890017	0.668222
T1	p	Young	G0	10	9.08125	3.023299	0.956051
T1	p	Young	G1	10	7.896333	2.031052	0.642275
T1	p	Young	G2	10	8.94775	2.191917	0.693145
T1	p	Young	G3	10	9.091	2.056069	0.650186
T1	p	Young	G4	10	8.401167	1.77406	0.561007
T1	p	Young	G5	10	9.064	1.751206	0.55378
T10	m	Old	G0	8	9.09875	1.686875	0.5964
T10	m	Old	G1	8	8.189063	1.51962	0.537267
T10	m	Old	G2	8	10.11031	3.360541	1.188131
T10	m	Old	G3	8	10.50094	3.656655	1.292823
T10	m	Old	G4	8	10.2175	3.437027	1.215173
T10	m	Old	G5	8	10.08156	3.491995	1.234607
T10	m	Young	G0	10	9.8455	2.539218	0.802971
T10	m	Young	G1	10	9.80475	1.419508	0.448888
T10	m	Young	G2	10	10.1705	1.520412	0.480796
T10	m	Young	G3	10	10.5165	1.973447	0.624059
T10	m	Young	G4	10	10.65275	2.320381	0.733769
T10	m	Young	G5	10	9.9475	2.005165	0.634089
T10	p	Old	G0	8	11.06531	3.750325	1.32594
T10	p	Old	G1	8	9.68625	1.708949	0.604205
T10	p	Old	G2	8	9.02375	5.014402	1.772859
T10	p	Old	G3	8	10.37813	4.409271	1.558913
T10	p	Old	G4	8	11.32594	3.402898	1.203106

T10	p	Old	G5	8	T10	3.609652	1.276205
T10	p	Young	G0	10	10.90575	1.612546	0.509932
T10	p	Young	G1	10	10.38383	1.9843	0.627491
T10	p	Young	G2	10	10.6585	2.191398	0.692981
T10	p	Young	G3	10	11.496	2.128313	0.673032
T10	p	Young	G4	10	10.7685	2.090845	0.661183
T10	p	Young	G5	10	11.5505	2.521574	0.797392
T2	m	Old	G0	8	7.755625	1.468701	0.519264
T2	m	Old	G1	8	8.447188	1.704141	0.602505
T2	m	Old	G2	8	8.224063	1.112024	0.39316
T2	m	Old	G3	8	8.265313	1.295493	0.458026
T2	m	Old	G4	8	9.211875	1.378651	0.487427
T2	m	Old	G5	8	7.895938	1.275173	0.450842
T2	m	Young	G0	10	10.1095	2.536513	0.802116
T2	m	Young	G1	10	9.99225	2.015412	0.637329
T2	m	Young	G2	10	8.9935	2.125559	0.672161
T2	m	Young	G3	10	10.097	2.396911	0.75797
T2	m	Young	G4	10	9.78775	2.199435	0.695523
T2	m	Young	G5	10	9.45	2.381219	0.753007
T2	p	Old	G0	8	9.178438	1.432183	0.506353
T2	p	Old	G1	8	9.8075	1.759644	0.622128
T2	p	Old	G2	8	8.9475	1.209241	0.427531
T2	p	Old	G3	8	8.463125	1.090288	0.385475
T2	p	Old	G4	8	9.593438	1.575951	0.557183
T2	p	Old	G5	8	9.053125	2.032786	0.718698
T2	p	Young	G0	10	10.29575	2.728001	0.86267
T2	p	Young	G1	10	9.276333	1.646669	0.520723
T2	p	Young	G2	10	10.25775	2.617889	0.827849
T2	p	Young	G3	10	10.3615	2.052213	0.648967
T2	p	Young	G4	10	9.556667	2.076365	0.656604
T2	p	Young	G5	10	10.293	1.844822	0.583384
T3	m	Old	G0	8	7.89625	2.021401	0.714673
T3	m	Old	G1	8	7.772813	2.294657	0.811284
T3	m	Old	G2	8	8.269688	1.95821	0.692332
T3	m	Old	G3	8	8.554063	1.85468	0.655728
T3	m	Old	G4	8	8.77375	1.993209	0.704706
T3	m	Old	G5	8	8.090313	2.10513	0.744276
T3	m	Young	G0	10	11.0145	2.164913	0.684606
T3	m	Young	G1	10	10.55575	1.95821	0.61924
T3	m	Young	G2	10	9.925	1.792252	0.56676
T3	m	Young	G3	10	10.2945	1.483224	0.469037
T3	m	Young	G4	10	10.68975	1.163587	0.367959
T3	m	Young	G5	10	9.94	1.124604	0.355631

T3	p	Old	G0	8	9.074063	1.897735	0.670951
T3	p	Old	G1	8	9.184375	1.281598	0.453113
T3	p	Old	G2	8	8.99625	2.149596	0.759997
T3	p	Old	G3	8	8.39	1.862063	0.658339
T3	p	Old	G4	8	9.537813	2.334903	0.825513
T3	p	Old	G5	8	9.121875	2.114687	0.747655
T3	p	Young	G0	10	10.60425	2.534156	0.80137
T3	p	Young	G1	10	9.516833	1.743656	0.551392
T3	p	Young	G2	10	9.83075	2.069872	0.654551
T3	p	Young	G3	10	10.9535	1.829427	0.578515
T3	p	Young	G4	10	10.10317	1.165976	0.368714
T3	p	Young	G5	10	10.8295	1.265121	0.400066
T4	m	Old	G0	8	7.701875	1.805497	0.63834
T4	m	Old	G1	8	7.399688	2.139359	0.756378
T4	m	Old	G2	8	8.443438	2.05166	0.725371
T4	m	Old	G3	8	8.002813	1.746123	0.617348
T4	m	Old	G4	8	7.729375	1.953148	0.690542
T4	m	Old	G5	8	7.535938	1.866581	0.659936
T4	m	Young	G0	10	8.34	1.114012	0.352282
T4	m	Young	G1	10	8.34375	1.079196	0.341272
T4	m	Young	G2	10	8.2235	1.012681	0.320238
T4	m	Young	G3	10	8.312	0.912251	0.288479
T4	m	Young	G4	10	8.43375	1.242738	0.392988
T4	m	Young	G5	10	8.0335	0.899078	0.284314
T4	p	Old	G0	8	7.936563	1.870588	0.661353
T4	p	Old	G1	8	8.718125	3.14249	1.111038
T4	p	Old	G2	8	8.408125	1.319207	0.46641
T4	p	Old	G3	8	7.930625	1.529287	0.540684
T4	p	Old	G4	8	8.289063	1.984991	0.7018
T4	p	Old	G5	8	7.845625	2.70702	0.957076
T4	p	Young	G0	10	8.71825	1.925461	0.608884
T4	p	Young	G1	10	8.012833	1.554943	0.491716
T4	p	Young	G2	10	8.47925	1.359795	0.430005
T4	p	Young	G3	10	8.682	1.181212	0.373532
T4	p	Young	G4	10	8.185167	0.739896	0.233976
T4	p	Young	G5	10	8.6655	1.29395	0.409183
T5	m	Old	G0	8	10.62188	4.559933	1.61218
T5	m	Old	G1	8	9.328438	4.94718	1.749092
T5	m	Old	G2	8	9.562188	4.283561	1.514468
T5	m	Old	G3	8	9.949063	3.781303	1.336893
T5	m	Old	G4	8	10.08938	3.228824	1.141562
T5	m	Old	G5	8	9.617188	2.977025	1.052537
T5	m	Young	G0	10	8.8585	2.277717	0.720277

T5	m	Young	G1	10	8.42325	2.993048	0.946485
T5	m	Young	G2	10	8.682	3.575808	1.13077
T5	m	Young	G3	10	8.791	3.275467	1.035794
T5	m	Young	G4	10	8.48975	3.000295	0.948777
T5	m	Young	G5	10	8.0705	3.365045	1.064121
T5	p	Old	G0	8	10.55531	4.713927	1.666625
T5	p	Old	G1	8	10.5275	4.666899	1.649998
T5	p	Old	G2	8	9.341875	2.679818	0.947459
T5	p	Old	G3	8	9.806875	3.149544	1.113532
T5	p	Old	G4	8	10.56344	4.124695	1.4583
T5	p	Old	G5	8	10.12688	3.128123	1.105958
T5	p	Young	G0	10	8.39375	1.850093	0.585051
T5	p	Young	G1	10	8.208333	2.879295	0.910513
T5	p	Young	G2	10	8.57875	3.056372	0.96651
T5	p	Young	G3	10	8.902	3.150486	0.996271
T5	p	Young	G4	10	8.711667	3.290618	1.040585
T5	p	Young	G5	10	8.91	2.456556	0.776831
T6	m	Old	G0	8	13.215	5.231245	1.849524
T6	m	Old	G1	8	12.70719	5.062936	1.790018
T6	m	Old	G2	8	12.30094	3.483881	1.231738
T6	m	Old	G3	8	12.58906	3.170677	1.121004
T6	m	Old	G4	8	13.40438	1.989712	0.703469
T6	m	Old	G5	8	12.34844	2.602413	0.920092
T6	m	Young	G0	10	14.207	3.944451	1.247345
T6	m	Young	G1	10	13.46175	3.845842	1.216162
T6	m	Young	G2	10	12.714	4.561783	1.442562
T6	m	Young	G3	10	12.805	3.66437	1.158776
T6	m	Young	G4	10	12.61075	3.460832	1.094411
T6	m	Young	G5	10	12.7915	3.651717	1.154774
T6	p	Old	G0	8	14.32406	3.853325	1.362356
T6	p	Old	G1	8	13.97813	3.079557	1.088788
T6	p	Old	G2	8	12.97563	2.136828	0.755483
T6	p	Old	G3	8	12.11688	3.344689	1.182526
T6	p	Old	G4	8	13.37969	2.718662	0.961192
T6	p	Old	G5	8	12.55313	4.055429	1.433811
T6	p	Young	G0	10	14.00675	4.181181	1.322206
T6	p	Young	G1	10	13.08425	4.370437	1.382054
T6	p	Young	G2	10	12.73075	4.469744	1.413457
T6	p	Young	G3	10	13.391	3.646745	1.153202
T6	p	Young	G4	10	12.57367	3.28243	1.037996
T6	p	Young	G5	10	13.6925	4.405534	1.393152
T7	m	Old	G0	8	11.30938	2.289426	0.809434
T7	m	Old	G1	8	10.21344	3.177479	1.123409

T7	m	Old	G2	8	11.27281	1.859921	0.657582
T7	m	Old	G3	8	13.31344	3.629403	1.283188
T7	m	Old	G4	8	12.31875	2.175442	0.769135
T7	m	Old	G5	8	10.66906	2.794678	0.988068
T7	m	Young	G0	10	14.20542	2.028197	0.641372
T7	m	Young	G1	10	12.87375	2.318036	0.733027
T7	m	Young	G2	10	12.997	3.036383	0.960189
T7	m	Young	G3	10	13.5355	2.887674	0.913163
T7	m	Young	G4	10	14.13175	2.268913	0.717493
T7	m	Young	G5	10	12.6185	2.527516	0.799271
T7	p	Old	G0	8	12.27406	2.920186	1.032442
T7	p	Old	G1	8	11.81938	2.571774	0.90926
T7	p	Old	G2	8	10.02813	1.767974	0.625073
T7	p	Old	G3	8	12.00063	1.884592	0.666304
T7	p	Old	G4	8	12.61656	2.421785	0.85623
T7	p	Old	G5	8	11.40063	3.663645	1.295294
T7	p	Young	G0	10	13.67475	2.046724	0.647231
T7	p	Young	G1	10	13.33033	2.296558	0.726235
T7	p	Young	G2	10	13.40725	3.45317	1.091988
T7	p	Young	G3	10	13.529	2.098576	0.663628
T7	p	Young	G4	10	13.315	2.504363	0.791949
T7	p	Young	G5	10	13.377	2.099762	0.664003
T8	m	Old	G0	8	10.54313	3.852138	1.361936
T8	m	Old	G1	8	10.29031	5.217109	1.844527
T8	m	Old	G2	8	11.28406	4.482366	1.584756
T8	m	Old	G3	8	11.71781	4.928662	1.742545
T8	m	Old	G4	8	10.2775	4.279874	1.513164
T8	m	Old	G5	8	10.85781	3.790039	1.339981
T8	m	Young	G0	10	9.393917	2.046949	0.647302
T8	m	Young	G1	10	9.65675	3.079764	0.973907
T8	m	Young	G2	10	9.605	3.395667	1.073804
T8	m	Young	G3	10	9.7685	3.278183	1.036652
T8	m	Young	G4	10	11.04625	3.151778	0.99668
T8	m	Young	G5	10	9.664	3.604649	1.13989
T8	p	Old	G0	8	12.42094	4.062119	1.436176
T8	p	Old	G1	8	11.3975	4.277993	1.512499
T8	p	Old	G2	8	9.9725	4.915115	1.737756
T8	p	Old	G3	8	9.653125	4.5341	1.603046
T8	p	Old	G4	8	11.50719	4.053587	1.433159
T8	p	Old	G5	8	11.32563	4.275882	1.511753
T8	p	Young	G0	10	9.80925	2.603355	0.823253
T8	p	Young	G1	10	9.988333	3.271855	1.034651
T8	p	Young	G2	10	10.685	3.088809	0.976767

T8	p	Young	G3	10	9.7575	2.972013	0.939833
T8	p	Young	G4	10	10.356	3.400265	1.075258
T8	p	Young	G5	10	10.6415	3.401636	1.075692
T9	m	Old	G0	8	17.04438	4.886243	1.727548
T9	m	Old	G1	8	16.83656	4.52649	1.600356
T9	m	Old	G2	8	17.16594	4.013542	1.419002
T9	m	Old	G3	8	17.33219	3.915504	1.38434
T9	m	Old	G4	8	17.70375	4.323875	1.528721
T9	m	Old	G5	8	17.27156	4.040189	1.428423
T9	m	Young	G0	10	17.7975	3.979194	1.258332
T9	m	Young	G1	10	17.85525	3.82958	1.21102
T9	m	Young	G2	10	17.9195	4.272926	1.351218
T9	m	Young	G3	10	18.106	4.4888	1.419483
T9	m	Young	G4	10	18.27925	4.007363	1.26724
T9	m	Young	G5	10	17.1895	3.833605	1.212292
T9	p	Old	G0	8	17.81844	3.599971	1.272782
T9	p	Old	G1	8	16.87313	3.815419	1.348954
T9	p	Old	G2	8	15.16063	4.761308	1.683376
T9	p	Old	G3	8	16.45625	4.412204	1.55995
T9	p	Old	G4	8	17.34219	4.280509	1.513389
T9	p	Old	G5	8	17.61063	5.11355	1.807913
T9	p	Young	G0	10	18.16725	4.299977	1.359772
T9	p	Young	G1	10	19.11133	4.059584	1.283753
T9	p	Young	G2	10	19.0815	4.093301	1.294416
T9	p	Young	G3	10	18.7545	3.969958	1.255411
T9	p	Young	G4	10	19.358	4.22257	1.335294
T9	p	Young	G5	10	18.884	3.986006	1.260486

Sum status: Values need to plot mean and standared error (SE)

Test 1: ANOVA F-test for each variable for main effect and interactions

Gene	Effect	NumDF	DenDF	FValue	Rawp
T1	AgeCat	1	16	2.37602	142755
T1	Trt	5	176	910692	475393
T1	AgeCat*Trt	5	176	721891	131851
T1	Glucose	1	176	439197	120133
T1	AgeCat*Glucose	1	176	634391	0.20278
T1	Trt*Glucose	5	176	636984	671763
T1	AgeCat*Trt*Glucose	5	176	347248	883516
T10	AgeCat	1	16	285762	0.6003
T10	Trt	5	176	814166	017986
T10	AgeCat*Trt	5	176	0.56564	726249
T10	Glucose	1	176	273752	002682
T10	AgeCat*Glucose	1	176	207892	648987
T10	Trt*Glucose	5	176	198915	311639
T10	AgeCat*Trt*Glucose	5	176	268587	279549
T2	AgeCat	1	16	2.76536	115787
T2	Trt	5	176	453577	810294
T2	AgeCat*Trt	5	176	034487	076006
T2	Glucose	1	176	415666	002492
T2	AgeCat*Glucose	1	176	644043	105728
T2	Trt*Glucose	5	176	0.80871	544862
T2	AgeCat*Trt*Glucose	5	176	061134	383645
T3	AgeCat	1	16	826155	018848
T3	Trt	5	176	915432	472299
T3	AgeCat*Trt	5	176	084312	370724
T3	Glucose	1	176	153045	043057
T3	AgeCat*Glucose	1	176	660278	010674
T3	Trt*Glucose	5	176	501396	774926
T3	AgeCat*Trt*Glucose	5	176	732703	0.12941
T4	AgeCat	1	16	0.62817	439622
T4	Trt	5	176	286636	919945
T4	AgeCat*Trt	5	176	461974	804162
T4	Glucose	1	176	234234	136774
T4	AgeCat*Glucose	1	176	311307	577588
T4	Trt*Glucose	5	176	136149	983771
T4	AgeCat*Trt*Glucose	5	176	828934	530663
T5	AgeCat	1	16	0.92698	349975
T5	Trt	5	176	772489	570759
T5	AgeCat*Trt	5	176	759249	580364
T5	Glucose	1	176	777059	379244
T5	AgeCat*Glucose	1	176	314845	575436

T5	Trt*Glucose	5	176	0.5878	0.70932
T5	AgeCat*Trt*Glucose	5	176	0.425858	0.830242
T6	AgeCat	1	16	0.015211	0.90338
T6	Trt	5	176	1.557367	0.174533
T6	AgeCat*Trt	5	176	0.599434	0.700426
T6	Glucose	1	176	0.951897	0.330576
T6	AgeCat*Glucose	1	176	0.250619	0.617265
T6	Trt*Glucose	5	176	0.095906	0.992734
T6	AgeCat*Trt*Glucose	5	176	0.504126	0.772879
T7	AgeCat	1	16	4.441972	0.051192
T7	Trt	5	176	3.111308	0.010223
T7	AgeCat*Trt	5	176	0.946109	0.452589
T7	Glucose	1	176	0.174962	0.676249
T7	AgeCat*Glucose	1	176	0.060257	0.806376
T7	Trt*Glucose	5	176	1.1044	0.35979
T7	AgeCat*Trt*Glucose	5	176	1.112324	0.355545
T8	AgeCat	1	16	0.376982	0.547854
T8	Trt	5	176	0.329639	0.894587
T8	AgeCat*Trt	5	176	0.619663	0.68497
T8	Glucose	1	176	0.907375	0.342118
T8	AgeCat*Glucose	1	176	0.049574	0.824065
T8	Trt*Glucose	5	176	1.136541	0.342809
T8	AgeCat*Trt*Glucose	5	176	1.54724	0.177527
T9	AgeCat	1	16	0.558556	0.465685
T9	Trt	5	176	0.639751	0.669656
T9	AgeCat*Trt	5	176	1.018661	0.408174
T9	Glucose	1	176	1.568225	0.212126
T9	AgeCat*Glucose	1	176	6.379704	0.012426
T9	Trt*Glucose	5	176	0.618324	0.685993
T9	AgeCat*Trt*Glucose	5	176	0.713982	0.613702

TEST 2: gaves comparisons of treatments (averaging over age for each gene and glucose level separately:

gene	Stm No	Effect	Slice	Trt	Trt	Estimate	StdErr	DF	tValue	Rawp	Adjustment	Adjp
T1	1	Trt*Gluc	Gluc m	G0	G1	-0.116	0.424	176	-0.274	0.784	Tukey-K	1.000
T1	1	Trt*Gluc	Gluc m	G0	G2	-0.092	0.424	176	-0.217	0.828	Tukey-K	1.000
T1	1	Trt*Gluc	Gluc m	G0	G3	-0.462	0.424	176	-1.090	0.277	Tukey-K	0.885
T1	1	Trt*Gluc	Gluc m	G0	G4	-0.551	0.424	176	-1.300	0.195	Tukey-K	0.785
T1	1	Trt*Gluc	Gluc m	G0	G5	0.074	0.424	176	0.176	0.861	Tukey-K	1.000
T1	1	Trt*Gluc	Gluc m	G1	G2	0.024	0.424	176	0.057	0.955	Tukey-K	1.000
T1	1	Trt*Gluc	Gluc m	G1	G3	-0.346	0.424	176	-0.816	0.416	Tukey-K	0.964
T1	1	Trt*Gluc	Gluc m	G1	G4	-0.434	0.424	176	-1.026	0.307	Tukey-K	0.909
T1	1	Trt*Gluc	Gluc m	G1	G5	0.190	0.424	176	0.450	0.653	Tukey-K	0.998
T1	1	Trt*Gluc	Gluc m	G2	G3	-0.370	0.424	176	-0.873	0.384	Tukey-K	0.952
T1	1	Trt*Gluc	Gluc m	G2	G4	-0.458	0.424	176	-1.082	0.281	Tukey-K	0.888
T1	1	Trt*Gluc	Gluc m	G2	G5	0.166	0.424	176	0.393	0.695	Tukey-K	0.999
T1	1	Trt*Gluc	Gluc m	G3	G4	-0.089	0.424	176	-0.209	0.834	Tukey-K	1.000
T1	1	Trt*Gluc	Gluc m	G3	G5	0.536	0.424	176	1.266	0.207	Tukey-K	0.803
T1	1	Trt*Gluc	Gluc m	G4	G5	0.625	0.424	176	1.475	0.142	Tukey-K	0.680
T1	1	Trt*Gluc	Gluc p	G0	G1	0.559	0.424	176	1.321	0.188	Tukey-K	0.773
T1	1	Trt*Gluc	Gluc p	G0	G2	-0.080	0.424	176	-0.188	0.851	Tukey-K	1.000
T1	1	Trt*Gluc	Gluc p	G0	G3	-0.039	0.424	176	-0.092	0.927	Tukey-K	1.000
T1	1	Trt*Gluc	Gluc p	G0	G4	-0.162	0.424	176	-0.384	0.702	Tukey-K	0.999
T1	1	Trt*Gluc	Gluc p	G0	G5	-0.157	0.424	176	-0.370	0.712	Tukey-K	0.999
T1	1	Trt*Gluc	Gluc p	G1	G2	-0.639	0.424	176	-1.509	0.133	Tukey-K	0.659
T1	1	Trt*Gluc	Gluc p	G1	G3	-0.598	0.424	176	-1.412	0.160	Tukey-K	0.719
T1	1	Trt*Gluc	Gluc p	G1	G4	-0.722	0.424	176	-1.704	0.090	Tukey-K	0.531
T1	1	Trt*Gluc	Gluc p	G1	G5	-0.716	0.424	176	-1.690	0.093	Tukey-K	0.540
T1	1	Trt*Gluc	Gluc p	G2	G3	0.041	0.424	176	0.096	0.923	Tukey-K	1.000
T1	1	Trt*Gluc	Gluc p	G2	G4	-0.083	0.424	176	-0.195	0.845	Tukey-K	1.000
T1	1	Trt*Gluc	Gluc p	G2	G5	-0.077	0.424	176	-0.181	0.856	Tukey-K	1.000
T1	1	Trt*Gluc	Gluc p	G3	G4	-0.124	0.424	176	-0.292	0.771	Tukey-K	1.000
T1	1	Trt*Gluc	Gluc p	G3	G5	-0.118	0.424	176	-0.278	0.781	Tukey-K	1.000
T1	1	Trt*Gluc	Gluc p	G4	G5	0.006	0.424	176	0.014	0.989	Tukey-K	1.000
T10	1	Trt*Gluc	Gluc m	G0	G1	0.475	0.563	176	0.845	0.399	Tukey-K	0.959
T10	1	Trt*Gluc	Gluc m	G0	G2	-0.668	0.563	176	-1.188	0.237	Tukey-K	0.842
T10	1	Trt*Gluc	Gluc m	G0	G3	-1.037	0.563	176	-1.842	0.067	Tukey-K	0.441
T10	1	Trt*Gluc	Gluc m	G0	G4	-0.963	0.563	176	-1.711	0.089	Tukey-K	0.526

T10	1	Trt*Gluc	Gluc m	G0	G5	-0.542	0.563	176	-0.964	0.336	Tukey-K	0.929
T10	1	Trt*Gluc	Gluc m	G1	G2	-1.144	0.563	176	-2.032	0.044	Tukey-K	0.328
T10	1	Trt*Gluc	Gluc m	G1	G3	-1.512	0.563	176	-2.687	0.008	Tukey-K	0.083
T10	1	Trt*Gluc	Gluc m	G1	G4	-1.438	0.563	176	-2.556	0.011	Tukey-K	0.114
T10	1	Trt*Gluc	Gluc m	G1	G5	-1.018	0.563	176	-1.809	0.072	Tukey-K	0.463
T10	1	Trt*Gluc	Gluc m	G2	G3	-0.368	0.563	176	-0.655	0.514	Tukey-K	0.986
T10	1	Trt*Gluc	Gluc m	G2	G4	-0.295	0.563	176	-0.524	0.601	Tukey-K	0.995
T10	1	Trt*Gluc	Gluc m	G2	G5	0.126	0.563	176	0.224	0.823	Tukey-K	1.000
T10	1	Trt*Gluc	Gluc m	G3	G4	0.074	0.563	176	0.131	0.896	Tukey-K	1.000
T10	1	Trt*Gluc	Gluc m	G3	G5	0.494	0.563	176	0.878	0.381	Tukey-K	0.951
T10	1	Trt*Gluc	Gluc m	G4	G5	0.421	0.563	176	0.747	0.456	Tukey-K	0.976
T10	1	Trt*Gluc	Gluc p	G0	G1	0.950	0.563	176	1.689	0.093	Tukey-K	0.541
T10	1	Trt*Gluc	Gluc p	G0	G2	1.144	0.563	176	2.034	0.043	Tukey-K	0.327
T10	1	Trt*Gluc	Gluc p	G0	G3	0.048	0.563	176	0.086	0.931	Tukey-K	1.000
T10	1	Trt*Gluc	Gluc p	G0	G4	-0.062	0.563	176	-0.110	0.913	Tukey-K	1.000
T10	1	Trt*Gluc	Gluc p	G0	G5	0.067	0.563	176	0.118	0.906	Tukey-K	1.000
T10	1	Trt*Gluc	Gluc p	G1	G2	0.194	0.563	176	0.345	0.731	Tukey-K	0.999
T10	1	Trt*Gluc	Gluc p	G1	G3	-0.902	0.563	176	-1.603	0.111	Tukey-K	0.598
T10	1	Trt*Gluc	Gluc p	G1	G4	-1.012	0.563	176	-1.799	0.074	Tukey-K	0.469
T10	1	Trt*Gluc	Gluc p	G1	G5	-0.884	0.563	176	-1.571	0.118	Tukey-K	0.619
T10	1	Trt*Gluc	Gluc p	G2	G3	-1.096	0.563	176	-1.948	0.053	Tukey-K	0.377
T10	1	Trt*Gluc	Gluc p	G2	G4	-1.206	0.563	176	-2.144	0.033	Tukey-K	0.270
T10	1	Trt*Gluc	Gluc p	G2	G5	-1.078	0.563	176	-1.916	0.057	Tukey-K	0.396
T10	1	Trt*Gluc	Gluc p	G3	G4	-0.110	0.563	176	-0.196	0.845	Tukey-K	1.000
T10	1	Trt*Gluc	Gluc p	G3	G5	0.018	0.563	176	0.032	0.974	Tukey-K	1.000
T10	1	Trt*Gluc	Gluc p	G4	G5	0.128	0.563	176	0.228	0.820	Tukey-K	1.000
T2	1	Trt*Gluc	Gluc m	G0	G1	-0.287	0.456	176	-0.630	0.530	Tukey-K	0.989
T2	1	Trt*Gluc	Gluc m	G0	G2	0.324	0.456	176	0.710	0.479	Tukey-K	0.981
T2	1	Trt*Gluc	Gluc m	G0	G3	-0.249	0.456	176	-0.545	0.586	Tukey-K	0.994
T2	1	Trt*Gluc	Gluc m	G0	G4	-0.567	0.456	176	-1.244	0.215	Tukey-K	0.814
T2	1	Trt*Gluc	Gluc m	G0	G5	0.260	0.456	176	0.569	0.570	Tukey-K	0.993
T2	1	Trt*Gluc	Gluc m	G1	G2	0.611	0.456	176	1.340	0.182	Tukey-K	0.762
T2	1	Trt*Gluc	Gluc m	G1	G3	0.039	0.456	176	0.085	0.933	Tukey-K	1.000
T2	1	Trt*Gluc	Gluc m	G1	G4	-0.280	0.456	176	-0.614	0.540	Tukey-K	0.990
T2	1	Trt*Gluc	Gluc m	G1	G5	0.547	0.456	176	1.199	0.232	Tukey-K	0.837
T2	1	Trt*Gluc	Gluc m	G2	G3	-0.572	0.456	176	-1.255	0.211	Tukey-K	0.809
T2	1	Trt*Gluc	Gluc m	G2	G4	-0.891	0.456	176	-1.954	0.052	Tukey-K	0.373

T2	1	Trt*Gluc	Gluc m	G2	G5	-0.064	0.456	176	-0.141	0.888	Tukey-K	1.000
T2	1	Trt*Gluc	Gluc m	G3	G4	-0.319	0.456	176	-0.699	0.486	Tukey-K	0.982
T2	1	Trt*Gluc	Gluc m	G3	G5	0.508	0.456	176	1.115	0.267	Tukey-K	0.875
T2	1	Trt*Gluc	Gluc m	G4	G5	0.827	0.456	176	1.813	0.071	Tukey-K	0.460
T2	1	Trt*Gluc	Gluc p	G0	G1	0.195	0.456	176	0.428	0.669	Tukey-K	0.998
T2	1	Trt*Gluc	Gluc p	G0	G2	0.134	0.456	176	0.295	0.768	Tukey-K	1.000
T2	1	Trt*Gluc	Gluc p	G0	G3	0.325	0.456	176	0.712	0.477	Tukey-K	0.980
T2	1	Trt*Gluc	Gluc p	G0	G4	0.162	0.456	176	0.355	0.723	Tukey-K	0.999
T2	1	Trt*Gluc	Gluc p	G0	G5	0.064	0.456	176	0.140	0.888	Tukey-K	1.000
T2	1	Trt*Gluc	Gluc p	G1	G2	-0.061	0.456	176	-0.133	0.894	Tukey-K	1.000
T2	1	Trt*Gluc	Gluc p	G1	G3	0.130	0.456	176	0.284	0.777	Tukey-K	1.000
T2	1	Trt*Gluc	Gluc p	G1	G4	-0.033	0.456	176	-0.073	0.942	Tukey-K	1.000
T2	1	Trt*Gluc	Gluc p	G1	G5	-0.131	0.456	176	-0.288	0.774	Tukey-K	1.000
T2	1	Trt*Gluc	Gluc p	G2	G3	0.190	0.456	176	0.417	0.677	Tukey-K	0.998
T2	1	Trt*Gluc	Gluc p	G2	G4	0.028	0.456	176	0.060	0.952	Tukey-K	1.000
T2	1	Trt*Gluc	Gluc p	G2	G5	-0.070	0.456	176	-0.154	0.877	Tukey-K	1.000
T2	1	Trt*Gluc	Gluc p	G3	G4	-0.163	0.456	176	-0.357	0.722	Tukey-K	0.999
T2	1	Trt*Gluc	Gluc p	G3	G5	-0.261	0.456	176	-0.572	0.568	Tukey-K	0.993
T2	1	Trt*Gluc	Gluc p	G4	G5	-0.098	0.456	176	-0.215	0.830	Tukey-K	1.000
T3	1	Trt*Gluc	Gluc m	G0	G1	0.291	0.437	176	0.666	0.507	Tukey-K	0.985
T3	1	Trt*Gluc	Gluc m	G0	G2	0.358	0.437	176	0.819	0.414	Tukey-K	0.964
T3	1	Trt*Gluc	Gluc m	G0	G3	0.031	0.437	176	0.071	0.943	Tukey-K	1.000
T3	1	Trt*Gluc	Gluc m	G0	G4	-0.276	0.437	176	-0.632	0.528	Tukey-K	0.988
T3	1	Trt*Gluc	Gluc m	G0	G5	0.440	0.437	176	1.007	0.315	Tukey-K	0.915
T3	1	Trt*Gluc	Gluc m	G1	G2	0.067	0.437	176	0.153	0.879	Tukey-K	1.000
T3	1	Trt*Gluc	Gluc m	G1	G3	-0.260	0.437	176	-0.595	0.553	Tukey-K	0.991
T3	1	Trt*Gluc	Gluc m	G1	G4	-0.567	0.437	176	-1.298	0.196	Tukey-K	0.786
T3	1	Trt*Gluc	Gluc m	G1	G5	0.149	0.437	176	0.341	0.734	Tukey-K	0.999
T3	1	Trt*Gluc	Gluc m	G2	G3	-0.327	0.437	176	-0.748	0.456	Tukey-K	0.976
T3	1	Trt*Gluc	Gluc m	G2	G4	-0.634	0.437	176	-1.451	0.149	Tukey-K	0.696
T3	1	Trt*Gluc	Gluc m	G2	G5	0.082	0.437	176	0.188	0.851	Tukey-K	1.000
T3	1	Trt*Gluc	Gluc m	G3	G4	-0.307	0.437	176	-0.703	0.483	Tukey-K	0.981
T3	1	Trt*Gluc	Gluc m	G3	G5	0.409	0.437	176	0.936	0.351	Tukey-K	0.937
T3	1	Trt*Gluc	Gluc m	G4	G5	0.717	0.437	176	1.639	0.103	Tukey-K	0.574
T3	1	Trt*Gluc	Gluc p	G0	G1	0.489	0.437	176	1.117	0.265	Tukey-K	0.874
T3	1	Trt*Gluc	Gluc p	G0	G2	0.426	0.437	176	0.973	0.332	Tukey-K	0.926
T3	1	Trt*Gluc	Gluc p	G0	G3	0.167	0.437	176	0.383	0.702	Tukey-K	0.999

T3	1	Trt*Gluc	Gluc p	G0	G4	0.019	0.437	176	0.043	0.966	Tukey-K	1.000
T3	1	Trt*Gluc	Gluc p	G0	G5	-0.137	0.437	176	-0.312	0.755	Tukey-K	1.000
T3	1	Trt*Gluc	Gluc p	G1	G2	-0.063	0.437	176	-0.144	0.886	Tukey-K	1.000
T3	1	Trt*Gluc	Gluc p	G1	G3	-0.321	0.437	176	-0.734	0.464	Tukey-K	0.977
T3	1	Trt*Gluc	Gluc p	G1	G4	-0.470	0.437	176	-1.074	0.284	Tukey-K	0.891
T3	1	Trt*Gluc	Gluc p	G1	G5	-0.625	0.437	176	-1.429	0.155	Tukey-K	0.709
T3	1	Trt*Gluc	Gluc p	G2	G3	-0.258	0.437	176	-0.591	0.556	Tukey-K	0.992
T3	1	Trt*Gluc	Gluc p	G2	G4	-0.407	0.437	176	-0.931	0.353	Tukey-K	0.938
T3	1	Trt*Gluc	Gluc p	G2	G5	-0.562	0.437	176	-1.286	0.200	Tukey-K	0.793
T3	1	Trt*Gluc	Gluc p	G3	G4	-0.149	0.437	176	-0.340	0.734	Tukey-K	0.999
T3	1	Trt*Gluc	Gluc p	G3	G5	-0.304	0.437	176	-0.695	0.488	Tukey-K	0.982
T3	1	Trt*Gluc	Gluc p	G4	G5	-0.155	0.437	176	-0.355	0.723	Tukey-K	0.999
T4	1	Trt*Gluc	Gluc m	G0	G1	0.149	0.460	176	0.324	0.746	Tukey-K	1.000
T4	1	Trt*Gluc	Gluc m	G0	G2	-0.313	0.460	176	-0.679	0.498	Tukey-K	0.984
T4	1	Trt*Gluc	Gluc m	G0	G3	-0.136	0.460	176	-0.296	0.767	Tukey-K	1.000
T4	1	Trt*Gluc	Gluc m	G0	G4	-0.061	0.460	176	-0.132	0.895	Tukey-K	1.000
T4	1	Trt*Gluc	Gluc m	G0	G5	0.236	0.460	176	0.513	0.609	Tukey-K	0.996
T4	1	Trt*Gluc	Gluc m	G1	G2	-0.462	0.460	176	-1.003	0.317	Tukey-K	0.916
T4	1	Trt*Gluc	Gluc m	G1	G3	-0.286	0.460	176	-0.620	0.536	Tukey-K	0.989
T4	1	Trt*Gluc	Gluc m	G1	G4	-0.210	0.460	176	-0.456	0.649	Tukey-K	0.997
T4	1	Trt*Gluc	Gluc m	G1	G5	0.087	0.460	176	0.189	0.850	Tukey-K	1.000
T4	1	Trt*Gluc	Gluc m	G2	G3	0.176	0.460	176	0.382	0.703	Tukey-K	0.999
T4	1	Trt*Gluc	Gluc m	G2	G4	0.252	0.460	176	0.547	0.585	Tukey-K	0.994
T4	1	Trt*Gluc	Gluc m	G2	G5	0.549	0.460	176	1.192	0.235	Tukey-K	0.840
T4	1	Trt*Gluc	Gluc m	G3	G4	0.076	0.460	176	0.165	0.869	Tukey-K	1.000
T4	1	Trt*Gluc	Gluc m	G3	G5	0.373	0.460	176	0.809	0.419	Tukey-K	0.965
T4	1	Trt*Gluc	Gluc m	G4	G5	0.297	0.460	176	0.645	0.520	Tukey-K	0.987
T4	1	Trt*Gluc	Gluc p	G0	G1	-0.038	0.460	176	-0.083	0.934	Tukey-K	1.000
T4	1	Trt*Gluc	Gluc p	G0	G2	-0.116	0.460	176	-0.253	0.801	Tukey-K	1.000
T4	1	Trt*Gluc	Gluc p	G0	G3	0.021	0.460	176	0.046	0.964	Tukey-K	1.000
T4	1	Trt*Gluc	Gluc p	G0	G4	0.090	0.460	176	0.196	0.845	Tukey-K	1.000
T4	1	Trt*Gluc	Gluc p	G0	G5	0.072	0.460	176	0.156	0.876	Tukey-K	1.000
T4	1	Trt*Gluc	Gluc p	G1	G2	-0.078	0.460	176	-0.170	0.865	Tukey-K	1.000
T4	1	Trt*Gluc	Gluc p	G1	G3	0.059	0.460	176	0.129	0.898	Tukey-K	1.000
T4	1	Trt*Gluc	Gluc p	G1	G4	0.128	0.460	176	0.279	0.781	Tukey-K	1.000
T4	1	Trt*Gluc	Gluc p	G1	G5	0.110	0.460	176	0.239	0.812	Tukey-K	1.000
T4	1	Trt*Gluc	Gluc p	G2	G3	0.137	0.460	176	0.298	0.766	Tukey-K	1.000

T4	1	Trt*Gluc	Gluc p	G2	G4	0.207	0.460	176	0.449	0.654	Tukey-K	0.998
T4	1	Trt*Gluc	Gluc p	G2	G5	0.188	0.460	176	0.409	0.683	Tukey-K	0.999
T4	1	Trt*Gluc	Gluc p	G3	G4	0.069	0.460	176	0.150	0.881	Tukey-K	1.000
T4	1	Trt*Gluc	Gluc p	G3	G5	0.051	0.460	176	0.110	0.912	Tukey-K	1.000
T4	1	Trt*Gluc	Gluc p	G4	G5	-0.018	0.460	176	-0.040	0.968	Tukey-K	1.000
T5	1	Trt*Gluc	Gluc m	G0	G1	0.864	0.496	176	1.742	0.083	Tukey-K	0.506
T5	1	Trt*Gluc	Gluc m	G0	G2	0.618	0.496	176	1.245	0.215	Tukey-K	0.814
T5	1	Trt*Gluc	Gluc m	G0	G3	0.370	0.496	176	0.746	0.457	Tukey-K	0.976
T5	1	Trt*Gluc	Gluc m	G0	G4	0.451	0.496	176	0.908	0.365	Tukey-K	0.944
T5	1	Trt*Gluc	Gluc m	G0	G5	0.896	0.496	176	1.806	0.073	Tukey-K	0.465
T5	1	Trt*Gluc	Gluc m	G1	G2	-0.246	0.496	176	-0.496	0.620	Tukey-K	0.996
T5	1	Trt*Gluc	Gluc m	G1	G3	-0.494	0.496	176	-0.996	0.321	Tukey-K	0.919
T5	1	Trt*Gluc	Gluc m	G1	G4	-0.414	0.496	176	-0.834	0.406	Tukey-K	0.961
T5	1	Trt*Gluc	Gluc m	G1	G5	0.032	0.496	176	0.064	0.949	Tukey-K	1.000
T5	1	Trt*Gluc	Gluc m	G2	G3	-0.248	0.496	176	-0.500	0.618	Tukey-K	0.996
T5	1	Trt*Gluc	Gluc m	G2	G4	-0.167	0.496	176	-0.337	0.736	Tukey-K	0.999
T5	1	Trt*Gluc	Gluc m	G2	G5	0.278	0.496	176	0.561	0.576	Tukey-K	0.993
T5	1	Trt*Gluc	Gluc m	G3	G4	0.080	0.496	176	0.162	0.871	Tukey-K	1.000
T5	1	Trt*Gluc	Gluc m	G3	G5	0.526	0.496	176	1.060	0.290	Tukey-K	0.896
T5	1	Trt*Gluc	Gluc m	G4	G5	0.446	0.496	176	0.898	0.370	Tukey-K	0.947
T5	1	Trt*Gluc	Gluc p	G0	G1	0.107	0.496	176	0.215	0.830	Tukey-K	1.000
T5	1	Trt*Gluc	Gluc p	G0	G2	0.514	0.496	176	1.036	0.302	Tukey-K	0.905
T5	1	Trt*Gluc	Gluc p	G0	G3	0.120	0.496	176	0.242	0.809	Tukey-K	1.000
T5	1	Trt*Gluc	Gluc p	G0	G4	-0.163	0.496	176	-0.328	0.743	Tukey-K	0.999
T5	1	Trt*Gluc	Gluc p	G0	G5	-0.044	0.496	176	-0.088	0.930	Tukey-K	1.000
T5	1	Trt*Gluc	Gluc p		G2	0.408	0.496	176	0.821	0.413	Tukey-K	0.963
T5	1	Trt*Gluc	Gluc p	G1	G3	0.013	0.496	176	0.027	0.978	Tukey-K	1.000
T5	1	Trt*Gluc	Gluc p	G1	G4	-0.270	0.496	176	-0.543	0.588	Tukey-K	0.994
T5	1	Trt*Gluc	Gluc p	G1	G5	-0.151	0.496	176	-0.303	0.762	Tukey-K	1.000
T5	1	Trt*Gluc	Gluc p	G2	G3	-0.394	0.496	176	-0.794	0.428	Tukey-K	0.968
T5	1	Trt*Gluc	Gluc p	G2	G4	-0.677	0.496	176	-1.365	0.174	Tukey-K	0.748
T5	1	Trt*Gluc	Gluc p	G2	G5	-0.558	0.496	176	-1.125	0.262	Tukey-K	0.871
T5	1	Trt*Gluc	Gluc p	G3	G4	-0.283	0.496	176	-0.570	0.569	Tukey-K	0.993
T5	1	Trt*Gluc	Gluc p	G3	G5	-0.164	0.496	176	-0.330	0.741	Tukey-K	0.999
T5	1	Trt*Gluc	Gluc p	G4	G5	0.119	0.496	176	0.240	0.811	Tukey-K	1.000
T6	1	Trt*Gluc	Gluc m	G0	G1	0.627	0.764	176	0.820	0.413	Tukey-K	0.964
T6	1	Trt*Gluc	Gluc m	G0	G2	1.204	0.764	176	1.575	0.117	Tukey-K	0.616

T6	1	Trt*Gluc	Gluc m	G0	G3	1.014	0.764	176	1.327	0.186	Tukey-K	0.770
T6	1	Trt*Gluc	Gluc m	G0	G4	0.703	0.764	176	0.921	0.358	Tukey-K	0.941
T6	1	Trt*Gluc	Gluc m	G0	G5	1.141	0.764	176	1.494	0.137	Tukey-K	0.669
T6	1	Trt*Gluc	Gluc m	G1	G2	0.577	0.764	176	0.755	0.451	Tukey-K	0.974
T6	1	Trt*Gluc	Gluc m	G1	G3	0.387	0.764	176	0.507	0.613	Tukey-K	0.996
T6	1	Trt*Gluc	Gluc m	G1	G4	0.077	0.764	176	0.101	0.920	Tukey-K	1.000
T6	1	Trt*Gluc	Gluc m	G1	G5	0.515	0.764	176	0.673	0.502	Tukey-K	0.985
T6	1	Trt*Gluc	Gluc m	G2	G3	-0.190	0.764	176	-0.248	0.804	Tukey-K	1.000
T6	1	Trt*Gluc	Gluc m	G2	G4	-0.500	0.764	176	-0.655	0.514	Tukey-K	0.986
T6	1	Trt*Gluc	Gluc m	G2	G5	-0.062	0.764	176	-0.082	0.935	Tukey-K	1.000
T6	1	Trt*Gluc	Gluc m	G3	G4	-0.311	0.764	176	-0.406	0.685	Tukey-K	0.999
T6	1	Trt*Gluc	Gluc m	G3	G5	0.127	0.764	176	0.166	0.868	Tukey-K	1.000
T6	1	Trt*Gluc	Gluc m	G4	G5	0.438	0.764	176	0.573	0.568	Tukey-K	0.993
T6	1	Trt*Gluc	Gluc p	G0	G1	0.634	0.764	176	0.830	0.408	Tukey-K	0.962
T6	1	Trt*Gluc	Gluc p	G0	G2	1.312	0.764	176	1.718	0.088	Tukey-K	0.522
T6	1	Trt*Gluc	Gluc p	G0	G3	1.411	0.764	176	1.848	0.066	Tukey-K	0.438
T6	1	Trt*Gluc	Gluc p	G0	G4	1.189	0.764	176	1.556	0.121	Tukey-K	0.628
T6	1	Trt*Gluc	Gluc p	G0	G5	1.043	0.764	176	1.365	0.174	Tukey-K	0.748
T6	1	Trt*Gluc	Gluc p	G1	G2	0.678	0.764	176	0.888	0.376	Tukey-K	0.949
T6	1	Trt*Gluc	Gluc p	G1	G3	0.777	0.764	176	1.017	0.310	Tukey-K	0.912
T6	1	Trt*Gluc	Gluc p	G1	G4	0.555	0.764	176	0.726	0.469	Tukey-K	0.979
T6	1	Trt*Gluc	Gluc p	G1	G5	0.408	0.764	176	0.535	0.594	Tukey-K	0.995
T6	1	Trt*Gluc	Gluc p	G2	G3	0.099	0.764	176	0.130	0.897	Tukey-K	1.000
T6	1	Trt*Gluc	Gluc p	G2	G4	-0.123	0.764	176	-0.162	0.872	Tukey-K	1.000
T6	1	Trt*Gluc	Gluc p	G2	G5	-0.270	0.764	176	-0.353	0.725	Tukey-K	0.999
T6	1	Trt*Gluc	Gluc p	G3	G4	-0.223	0.764	176	-0.292	0.771	Tukey-K	1.000
T6	1	Trt*Gluc	Gluc p	G3	G5	-0.369	0.764	176	-0.483	0.630	Tukey-K	0.997
T6	1	Trt*Gluc	Gluc p	G4	G5	-0.146	0.764	176	-0.191	0.849	Tukey-K	1.000
T7	1	Trt*Gluc	Gluc m	G0	G1	1.214	0.641	176	1.893	0.060	Tukey-K	0.410
T7	1	Trt*Gluc	Gluc m	G0	G2	0.622	0.641	176	0.971	0.333	Tukey-K	0.927
T7	1	Trt*Gluc	Gluc m	G0	G3	-0.667	0.641	176	-1.040	0.300	Tukey-K	0.904
T7	1	Trt*Gluc	Gluc m	G0	G4	-0.468	0.641	176	-0.730	0.467	Tukey-K	0.978
T7	1	Trt*Gluc	Gluc m	G0	G5	1.114	0.641	176	1.737	0.084	Tukey-K	0.509
T7	1	Trt*Gluc	Gluc m	G1	G2	-0.591	0.641	176	-0.922	0.358	Tukey-K	0.940
T7	1	Trt*Gluc	Gluc m	G1	G3	-1.881	0.641	176	-2.933	0.004	Tukey-K	0.043
T7	1	Trt*Gluc	Gluc m	G1	G4	-1.682	0.641	176	-2.623	0.009	Tukey-K	0.097
T7	1	Trt*Gluc	Gluc m	G1	G5	-0.100	0.641	176	-0.156	0.876	Tukey-K	1.000

T7	1	Trt*Gluc	Gluc m	G2	G3	-1.290	0.641	176	-2.011	0.046	Tukey-K	0.340
T7	1	Trt*Gluc	Gluc m	G2	G4	-1.090	0.641	176	-1.700	0.091	Tukey-K	0.533
T7	1	Trt*Gluc	Gluc m	G2	G5	0.491	0.641	176	0.766	0.445	Tukey-K	0.973
T7	1	Trt*Gluc	Gluc m	G3	G4	0.199	0.641	176	0.311	0.756	Tukey-K	1.000
T7	1	Trt*Gluc	Gluc m	G3	G5	1.781	0.641	176	2.777	0.006	Tukey-K	0.066
T7	1	Trt*Gluc	Gluc m	G4	G5	1.581	0.641	176	2.466	0.015	Tukey-K	0.140
T7	1	Trt*Gluc	Gluc p	G0	G1	0.400	0.641	176	0.623	0.534	Tukey-K	0.989
T7	1	Trt*Gluc	Gluc p	G0	G2	1.257	0.641	176	1.960	0.052	Tukey-K	0.370
T7	1	Trt*Gluc	Gluc p	G0	G3	0.210	0.641	176	0.327	0.744	Tukey-K	0.999
T7	1	Trt*Gluc	Gluc p	G0	G4	0.009	0.641	176	0.013	0.989	Tukey-K	1.000
T7	1	Trt*Gluc	Gluc p	G0	G5	0.586	0.641	176	0.913	0.362	Tukey-K	0.943
T7	1	Trt*Gluc	Gluc p	G1	G2	0.857	0.641	176	1.337	0.183	Tukey-K	0.764
T7	1	Trt*Gluc	Gluc p	G1	G3	-0.190	0.641	176	-0.296	0.767	Tukey-K	1.000
T7	1	Trt*Gluc	Gluc p	G1	G4	-0.391	0.641	176	-0.610	0.543	Tukey-K	0.990
T7	1	Trt*Gluc	Gluc p	G1	G5	0.186	0.641	176	0.290	0.772	Tukey-K	1.000
T7	1	Trt*Gluc	Gluc p	G2	G3	-1.047	0.641	176	-1.633	0.104	Tukey-K	0.578
T7	1	Trt*Gluc	Gluc p	G2	G4	-1.248	0.641	176	-1.947	0.053	Tukey-K	0.378
T7	1	Trt*Gluc	Gluc p	G2	G5	-0.671	0.641	176	-1.047	0.297	Tukey-K	0.901
T7	1	Trt*Gluc	Gluc p	G3	G4	-0.201	0.641	176	-0.313	0.754	Tukey-K	1.000
T7	1	Trt*Gluc	Gluc p	G3	G5	0.376	0.641	176	0.586	0.558	Tukey-K	0.992
T7	1	Trt*Gluc	Gluc p	G4	G5	0.577	0.641	176	0.900	0.369	Tukey-K	0.946
T8	1	Trt*Gluc	Gluc m	G0	G1	-0.005	0.731	176	-0.007	0.995	Tukey-K	1.000
T8	1	Trt*Gluc	Gluc m	G0	G2	-0.476	0.731	176	-0.652	0.516	Tukey-K	0.987
T8	1	Trt*Gluc	Gluc m	G0	G3	-0.775	0.731	176	-1.060	0.290	Tukey-K	0.896
T8	1	Trt*Gluc	Gluc m	G0	G4	-0.693	0.731	176	-0.949	0.344	Tukey-K	0.933
T8	1	Trt*Gluc	Gluc m	G0	G5	-0.292	0.731	176	-0.400	0.689	Tukey-K	0.999
T8	1	Trt*Gluc	Gluc m	G1	G2	-0.471	0.731	176	-0.645	0.520	Tukey-K	0.987
T8	1	Trt*Gluc	Gluc m	G1	G3	-0.770	0.731	176	-1.053	0.294	Tukey-K	0.899
T8	1	Trt*Gluc	Gluc m	G1	G4	-0.688	0.731	176	-0.942	0.347	Tukey-K	0.935
T8	1	Trt*Gluc	Gluc m	G1	G5	-0.287	0.731	176	-0.393	0.695	Tukey-K	0.999
T8	1	Trt*Gluc	Gluc m	G2	G3	-0.299	0.731	176	-0.409	0.683	Tukey-K	0.999
T8	1	Trt*Gluc	Gluc m	G2	G4	-0.217	0.731	176	-0.297	0.766	Tukey-K	1.000
T8	1	Trt*Gluc	Gluc m	G2	G5	0.184	0.731	176	0.251	0.802	Tukey-K	1.000
T8	1	Trt*Gluc	Gluc m	G3	G4	0.081	0.731	176	0.111	0.912	Tukey-K	1.000
T8	1	Trt*Gluc	Gluc m	G3	G5	0.482	0.731	176	0.660	0.510	Tukey-K	0.986
T8	1	Trt*Gluc	Gluc m	G4	G5	0.401	0.731	176	0.549	0.584	Tukey-K	0.994
T8	1	Trt*Gluc	Gluc p	G0	G1	0.422	0.731	176	0.578	0.564	Tukey-K	0.992

T8	1	Trt*Gluc	Gluc p	G0	G2	0.786	0.731	176	1.076	0.283	Tukey-K	0.890
T8	1	Trt*Gluc	Gluc p	G0	G3	1.410	0.731	176	1.930	0.055	Tukey-K	0.388
T8	1	Trt*Gluc	Gluc p	G0	G4	0.183	0.731	176	0.251	0.802	Tukey-K	1.000
T8	1	Trt*Gluc	Gluc p	G0	G5	0.132	0.731	176	0.180	0.857	Tukey-K	1.000
T8	1	Trt*Gluc	Gluc p	G1	G2	0.364	0.731	176	0.498	0.619	Tukey-K	0.996
T8	1	Trt*Gluc	Gluc p	G1	G3	0.988	0.731	176	1.352	0.178	Tukey-K	0.756
T8	1	Trt*Gluc	Gluc p	G1	G4	-0.239	0.731	176	-0.327	0.744	Tukey-K	0.999
T8	1	Trt*Gluc	Gluc p	G1	G5	-0.291	0.731	176	-0.398	0.691	Tukey-K	0.999
T8	1	Trt*Gluc	Gluc p	G2	G3	0.623	0.731	176	0.853	0.395	Tukey-K	0.957
T8	1	Trt*Gluc	Gluc p	G2	G4	-0.603	0.731	176	-0.825	0.410	Tukey-K	0.963
T8	1	Trt*Gluc	Gluc p	G2	G5	-0.655	0.731	176	-0.896	0.371	Tukey-K	0.947
T8	1	Trt*Gluc	Gluc p	G3	G4	-1.226	0.731	176	-1.678	0.095	Tukey-K	0.548
T8	1	Trt*Gluc	Gluc p	G3	G5	-1.278	0.731	176	-1.750	0.082	Tukey-K	0.501
T8	1	Trt*Gluc	Gluc p	G4	G5	-0.052	0.731	176	-0.071	0.943	Tukey-K	1.000
T9	1	Trt*Gluc	Gluc m	G0	G1	0.075	0.671	176	0.112	0.911	Tukey-K	1.000
T9	1	Trt*Gluc	Gluc m	G0	G2	-0.122	0.671	176	-0.181	0.856	Tukey-K	1.000
T9	1	Trt*Gluc	Gluc m	G0	G3	-0.298	0.671	176	-0.444	0.657	Tukey-K	0.998
T9	1	Trt*Gluc	Gluc m	G0	G4	-0.571	0.671	176	-0.850	0.396	Tukey-K	0.957
T9	1	Trt*Gluc	Gluc m	G0	G5	0.190	0.671	176	0.284	0.777	Tukey-K	1.000
T9	1	Trt*Gluc	Gluc m	G1	G2	-0.197	0.671	176	-0.293	0.770	Tukey-K	1.000
T9	1	Trt*Gluc	Gluc m	G1	G3	-0.373	0.671	176	-0.556	0.579	Tukey-K	0.994
T9	1	Trt*Gluc	Gluc m	G1	G4	-0.646	0.671	176	-0.962	0.337	Tukey-K	0.929
T9	1	Trt*Gluc	Gluc m	G1	G5	0.115	0.671	176	0.172	0.864	Tukey-K	1.000
T9	1	Trt*Gluc	Gluc m	G2	G3	-0.176	0.671	176	-0.263	0.793	Tukey-K	1.000
T9	1	Trt*Gluc	Gluc m	G2	G4	-0.449	0.671	176	-0.669	0.504	Tukey-K	0.985
T9	1	Trt*Gluc	Gluc m	G2	G5	0.312	0.671	176	0.465	0.642	Tukey-K	0.997
T9	1	Trt*Gluc	Gluc m	G3	G4	-0.272	0.671	176	-0.406	0.685	Tukey-K	0.999
T9	1	Trt*Gluc	Gluc m	G3	G5	0.489	0.671	176	0.728	0.468	Tukey-K	0.978
T9	1	Trt*Gluc	Gluc m	G4	G5	0.761	0.671	176	1.134	0.258	Tukey-K	0.867
T9	1	Trt*Gluc	Gluc p	G0	G1	0.001	0.671	176	0.001	0.999	Tukey-K	1.000
T9	1	Trt*Gluc	Gluc p	G0	G2	0.872	0.671	176	1.299	0.196	Tukey-K	0.785
T9	1	Trt*Gluc	Gluc p	G0	G3	0.387	0.671	176	0.577	0.564	Tukey-K	0.992
T9	1	Trt*Gluc	Gluc p	G0	G4	-0.357	0.671	176	-0.532	0.595	Tukey-K	0.995
T9	1	Trt*Gluc	Gluc p	G0	G5	-0.254	0.671	176	-0.379	0.705	Tukey-K	0.999
T9	1	Trt*Gluc	Gluc p	G1	G2	0.871	0.671	176	1.298	0.196	Tukey-K	0.786
T9	1	Trt*Gluc	Gluc p	G1	G3	0.387	0.671	176	0.577	0.565	Tukey-K	0.992
T9	1	Trt*Gluc	Gluc p	G1	G4	-0.358	0.671	176	-0.533	0.594	Tukey-K	0.995

T9	1	Trt*Gluc	Gluc p	G1	G5	-0.255	0.671	176	-0.380	0.704	Tukey-K	0.999
T9	1	Trt*Gluc	Gluc p	G2	G3	-0.484	0.671	176	-0.722	0.471	Tukey-K	0.979
T9	1	Trt*Gluc	Gluc p	G2	G4	-1.229	0.671	176	-1.832	0.069	Tukey-K	0.448
T9	1	Trt*Gluc	Gluc p	G2	G5	-1.126	0.671	176	-1.678	0.095	Tukey-K	0.548
T9	1	Trt*Gluc	Gluc p	G3	G4	-0.745	0.671	176	-1.110	0.269	Tukey-K	0.877
T9	1	Trt*Gluc	Gluc p	G3	G5	-0.642	0.671	176	-0.957	0.340	Tukey-K	0.931
T9	1	Trt*Gluc	Gluc p	G4	G5	0.103	0.671	176	0.153	0.878	Tukey-K	1.000

TEST 3: gives comparisons of ages (averaging over treatments) for each gene and glucose leve separately:

Gene	St No	Effect	Slice	AgeCat	_AgeCat	Estimate	StdErr	DF	tValue	Rawp	Adjust	Adj p
T1	2	AgeCat*Gluc	Gluc m	Old	Young	-1.361	0.759	17.79	-1.792	0.090	Tukey-K	0.075
T1	2	AgeCat*Gluc	Gluc p	Old	Young	-0.919	0.759	17.79	-1.210	0.242	Tukey-K	0.228
T10	2	AgeCat*Gluc	Gluc m	Old	Young	-0.457	1.075	17.56	-0.425	0.676	Tukey-K	0.672
T10	2	AgeCat*Gluc	Gluc p	Old	Young	-0.666	1.075	17.56	-0.620	0.543	Tukey-K	0.536
T2	2	AgeCat*Gluc	Gluc m	Old	Young	-1.438	0.708	18.46	-2.032	0.057	Tukey-K	0.044
T2	2	AgeCat*Gluc	Gluc p	Old	Young	-0.833	0.708	18.46	-1.177	0.254	Tukey-K	0.241
T3	2	AgeCat*Gluc	Gluc m	Old	Young	-2.177	0.681	18.44	-3.198	0.005	Tukey-K	0.002
T3	2	AgeCat*Gluc	Gluc p	Old	Young	-1.256	0.681	18.44	-1.844	0.081	Tukey-K	0.067
T4	2	AgeCat*Gluc	Gluc m	Old	Young	-0.479	0.508	21.43	-0.943	0.356	Tukey-K	0.347
T4	2	AgeCat*Gluc	Gluc p	Old	Young	-0.269	0.508	21.43	-0.530	0.602	Tukey-K	0.597
T5	2	AgeCat*Gluc	Gluc m	Old	Young	1.309	1.491	16.61	0.878	0.393	Tukey-K	0.381
T5	2	AgeCat*Gluc	Gluc p	Old	Young	1.536	1.491	16.61	1.030	0.318	Tukey-K	0.304
T6	2	AgeCat*Gluc	Gluc m	Old	Young	-0.338	1.503	17.47	-0.225	0.825	Tukey-K	0.823
T6	2	AgeCat*Gluc	Gluc p	Old	Young	-0.025	1.503	17.47	-0.017	0.987	Tukey-K	0.987
T7	2	AgeCat*Gluc	Gluc m	Old	Young	-1.878	0.899	19.08	-2.088	0.050	Tukey-K	0.038
T7	2	AgeCat*Gluc	Gluc p	Old	Young	-1.749	0.899	19.08	-1.945	0.067	Tukey-K	0.053
T8	2	AgeCat*Gluc	Gluc m	Old	Young	0.973	1.506	17.33	0.646	0.527	Tukey-K	0.519
T8	2	AgeCat*Gluc	Gluc p	Old	Young	0.840	1.506	17.33	0.558	0.584	Tukey-K	0.578
T9	2	AgeCat*Gluc	Gluc m	Old	Young	-0.632	1.793	16.77	-0.353	0.729	Tukey-K	0.725
T9	2	AgeCat*Gluc	Gluc p	Old	Young	-2.016	1.793	16.77	-1.125	0.277	Tukey-K	0.262

TEST 4: gives comparisions treatments for each geneand glucose level and age separately:

Gene	St No	Effect	Slice	Trt	Trt	Esti	StdErr	DF	tValue	Rawp	Adj	Adj p
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G1	-0.29	0.63	176	-0.45	0.651	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G2	-0.50	0.63	176	-0.80	0.425	Tukey	0.97
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G3	-0.72	0.63	176	-1.14	0.256	Tukey	0.86
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G4	-1.32	0.63	176	-2.09	0.038	Tukey	0.30
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G5	-0.18	0.63	176	-0.29	0.773	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G2	-0.22	0.63	176	-0.35	0.730	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G3	-0.43	0.63	176	-0.69	0.494	Tukey	0.98
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G4	-1.03	0.63	176	-1.64	0.104	Tukey	0.58
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G5	0.10	0.63	176	0.16	0.870	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G3	-0.22	0.63	176	-0.34	0.734	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G4	-0.81	0.63	176	-1.29	0.199	Tukey	0.79
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G5	0.32	0.63	176	0.51	0.611	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G4	-0.60	0.63	176	-0.95	0.344	Tukey	0.93
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G5	0.54	0.63	176	0.85	0.396	Tukey	0.96
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G4	G5	1.14	0.63	176	1.80	0.074	Tukey	0.47
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G1	-0.07	0.63	176	-0.10	0.917	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G2	-0.29	0.63	176	-0.46	0.643	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G3	-0.07	0.63	176	-0.11	0.915	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G4	-1.01	0.63	176	-1.59	0.113	Tukey	0.61
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G5	-0.33	0.63	176	-0.52	0.602	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G2	-0.23	0.63	176	-0.36	0.720	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G3	0.00	0.63	176	0.00	0.998	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G4	-0.94	0.63	176	-1.49	0.139	Tukey	0.67
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G5	-0.26	0.63	176	-0.42	0.676	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G3	0.22	0.63	176	0.36	0.722	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G4	-0.71	0.63	176	-1.13	0.261	Tukey	0.87
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G5	-0.04	0.63	176	-0.06	0.953	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G4	-0.94	0.63	176	-1.48	0.140	Tukey	0.67
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G5	-0.26	0.63	176	-0.42	0.678	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G4	G5	0.67	0.63	176	1.07	0.287	Tukey	0.89
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G1	0.05	0.56	176	0.10	0.924	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G2	0.32	0.56	176	0.57	0.571	Tukey	0.99
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G3	-0.20	0.56	176	-0.36	0.718	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G4	0.22	0.56	176	0.39	0.700	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G5	0.33	0.56	176	0.59	0.558	Tukey	0.99
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G2	0.27	0.56	176	0.47	0.638	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G3	-0.26	0.56	176	-0.46	0.648	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G4	0.16	0.56	176	0.29	0.772	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G5	0.28	0.56	176	0.49	0.624	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G3	-0.52	0.56	176	-0.93	0.354	Tukey	0.94

T1	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G4	-0.10	0.56	176	-0.18	0.857	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G5	0.01	0.56	176	0.02	0.984	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G4	0.42	0.56	176	0.75	0.456	Tukey	0.98
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G5	0.54	0.56	176	0.95	0.344	Tukey	0.93
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G4	G5	0.11	0.56	176	0.20	0.841	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G1	1.18	0.56	176	2.10	0.037	Tukey	0.29
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G2	0.13	0.56	176	0.24	0.813	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G3	-0.01	0.56	176	-0.02	0.986	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G4	0.68	0.56	176	1.20	0.230	Tukey	0.83
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G5	0.02	0.56	176	0.03	0.976	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G2	-1.05	0.56	176	-1.86	0.064	Tukey	0.43
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G3	-1.19	0.56	176	-2.12	0.036	Tukey	0.28
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G4	-0.50	0.56	176	-0.89	0.373	Tukey	0.95
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G5	-1.17	0.56	176	-2.07	0.040	Tukey	0.31
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G3	-0.14	0.56	176	-0.25	0.800	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G4	0.55	0.56	176	0.97	0.334	Tukey	0.93
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G5	-0.12	0.56	176	-0.21	0.837	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G4	0.69	0.56	176	1.22	0.224	Tukey	0.83
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G5	0.03	0.56	176	0.05	0.962	Tukey	1.00
T1	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G4	G5	-0.66	0.56	176	-1.17	0.242	Tukey	0.85
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G1	0.91	0.84	176	1.08	0.280	Tukey	0.89
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G2	-1.01	0.84	176	-1.21	0.229	Tukey	0.83
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G3	-1.40	0.84	176	-1.67	0.096	Tukey	0.55
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G4	-1.12	0.84	176	-1.33	0.184	Tukey	0.77
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G5	-0.98	0.84	176	-1.17	0.243	Tukey	0.85
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G2	-1.92	0.84	176	-2.29	0.023	Tukey	0.20
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G3	-2.31	0.84	176	-2.76	0.006	Tukey	0.07
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G4	-2.03	0.84	176	-2.42	0.017	Tukey	0.16
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G5	-1.89	0.84	176	-2.26	0.025	Tukey	0.22
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G3	-0.39	0.84	176	-0.47	0.642	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G4	-0.11	0.84	176	-0.13	0.898	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G5	0.03	0.84	176	0.03	0.973	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G4	0.28	0.84	176	0.34	0.736	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G5	0.42	0.84	176	0.50	0.618	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G4	G5	0.14	0.84	176	0.16	0.871	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G1	1.38	0.84	176	1.64	0.102	Tukey	0.57
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G2	2.04	0.84	176	2.43	0.016	Tukey	0.15
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G3	0.69	0.84	176	0.82	0.414	Tukey	0.96
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G4	-0.26	0.84	176	-0.31	0.756	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G5	0.78	0.84	176	0.93	0.355	Tukey	0.94
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G2	0.66	0.84	176	0.79	0.431	Tukey	0.97
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G3	-0.69	0.84	176	-0.82	0.411	Tukey	0.96
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G4	-1.64	0.84	176	-1.95	0.052	Tukey	0.37

T10	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G5	-0.60	0.84	176	-0.72	0.474	Tukey	0.98
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G3	-1.35	0.84	176	-1.61	0.108	Tukey	0.59
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G4	-2.30	0.84	176	-2.74	0.007	Tukey	0.07
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G5	-1.26	0.84	176	-1.51	0.134	Tukey	0.66
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G4	-0.95	0.84	176	-1.13	0.260	Tukey	0.87
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G5	0.09	0.84	176	0.11	0.914	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G4	G5	1.04	0.84	176	1.24	0.217	Tukey	0.82
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G1	0.04	0.75	176	0.05	0.957	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G2	-0.32	0.75	176	-0.43	0.665	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G3	-0.67	0.75	176	-0.89	0.372	Tukey	0.95
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G4	-0.81	0.75	176	-1.08	0.283	Tukey	0.89
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G5	-0.10	0.75	176	-0.14	0.892	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G2	-0.37	0.75	176	-0.49	0.626	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G3	-0.71	0.75	176	-0.95	0.344	Tukey	0.93
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G4	-0.85	0.75	176	-1.13	0.260	Tukey	0.87
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G5	-0.14	0.75	176	-0.19	0.849	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G3	-0.35	0.75	176	-0.46	0.645	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G4	-0.48	0.75	176	-0.64	0.521	Tukey	0.99
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G5	0.22	0.75	176	0.30	0.767	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G4	-0.14	0.75	176	-0.18	0.856	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G5	0.57	0.75	176	0.76	0.449	Tukey	0.97
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G4	G5	0.71	0.75	176	0.94	0.348	Tukey	0.94
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G1	0.52	0.75	176	0.70	0.488	Tukey	0.98
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G2	0.25	0.75	176	0.33	0.742	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G3	-0.59	0.75	176	-0.79	0.432	Tukey	0.97
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G4	0.14	0.75	176	0.18	0.855	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G5	-0.64	0.75	176	-0.86	0.391	Tukey	0.96
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G2	-0.27	0.75	176	-0.37	0.715	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G3	-1.11	0.75	176	-1.48	0.140	Tukey	0.68
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G4	-0.38	0.75	176	-0.51	0.609	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G5	-1.17	0.75	176	-1.56	0.122	Tukey	0.63
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G3	-0.84	0.75	176	-1.12	0.266	Tukey	0.87
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G4	-0.11	0.75	176	-0.15	0.884	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G5	-0.89	0.75	176	-1.19	0.236	Tukey	0.84
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G4	0.73	0.75	176	0.97	0.334	Tukey	0.93
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G5	-0.05	0.75	176	-0.07	0.942	Tukey	1.00
T10	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G4	G5	-0.78	0.75	176	-1.04	0.299	Tukey	0.90
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G1	-0.69	0.68	176	-1.02	0.310	Tukey	0.91
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G2	-0.47	0.68	176	-0.69	0.492	Tukey	0.98
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G3	-0.51	0.68	176	-0.75	0.454	Tukey	0.98
T2	3	eCat*Trt*Glu	AgeCat Old Glu m	G0	G4	-1.46	0.68	176	-2.14	0.034	Tukey	0.27
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G5	-0.14	0.68	176	-0.21	0.837	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G2	0.22	0.68	176	0.33	0.743	Tukey	1.00

T2	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G3	0.18	0.68	176	0.27	0.789	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G4	-0.76	0.68	176	-1.13	0.262	Tukey	0.87
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G5	0.55	0.68	176	0.81	0.418	Tukey	0.97
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G3	-0.04	0.68	176	-0.06	0.952	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G4	-0.99	0.68	176	-1.45	0.148	Tukey	0.69
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G5	0.33	0.68	176	0.48	0.630	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G4	-0.95	0.68	176	-1.39	0.165	Tukey	0.73
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G5	0.37	0.68	176	0.54	0.588	Tukey	0.99
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G4	G5	1.32	0.68	176	1.94	0.054	Tukey	0.38
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G1	-0.63	0.68	176	-0.93	0.356	Tukey	0.94
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G2	0.23	0.68	176	0.34	0.734	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G3	0.72	0.68	176	1.05	0.294	Tukey	0.90
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G4	-0.41	0.68	176	-0.61	0.542	Tukey	0.99
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G5	0.13	0.68	176	0.18	0.854	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G2	0.86	0.68	176	1.27	0.207	Tukey	0.80
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G3	1.34	0.68	176	1.98	0.050	Tukey	0.36
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G4	0.21	0.68	176	0.31	0.753	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G5	0.75	0.68	176	1.11	0.269	Tukey	0.88
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G3	0.48	0.68	176	0.71	0.477	Tukey	0.98
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G4	-0.65	0.68	176	-0.95	0.343	Tukey	0.93
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G5	-0.11	0.68	176	-0.16	0.877	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G4	-1.13	0.68	176	-1.66	0.098	Tukey	0.56
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G5	-0.59	0.68	176	-0.87	0.387	Tukey	0.95
T2	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G4	G5	0.54	0.68	176	0.79	0.428	Tukey	0.97
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G1	0.12	0.61	176	0.19	0.847	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G2	1.12	0.61	176	1.84	0.068	Tukey	0.45
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G3	0.01	0.61	176	0.02	0.984	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G4	0.32	0.61	176	0.53	0.597	Tukey	0.99
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G5	0.66	0.61	176	1.08	0.279	Tukey	0.89
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G2	1.00	0.61	176	1.64	0.102	Tukey	0.57
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G3	-0.10	0.61	176	-0.17	0.863	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G4	0.20	0.61	176	0.34	0.737	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G5	0.54	0.61	176	0.89	0.374	Tukey	0.95
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G3	-1.10	0.61	176	-1.82	0.071	Tukey	0.46
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G4	-0.79	0.61	176	-1.31	0.193	Tukey	0.78
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G5	-0.46	0.61	176	-0.75	0.454	Tukey	0.98
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G4	0.31	0.61	176	0.51	0.612	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G5	0.65	0.61	176	1.06	0.289	Tukey	0.89
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G4	G5	0.34	0.61	176	0.56	0.579	Tukey	0.99
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G1	1.02	0.61	176	1.68	0.095	Tukey	0.55
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G2	0.04	0.61	176	0.06	0.950	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G3	-0.07	0.61	176	-0.11	0.914	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G4	0.74	0.61	176	1.22	0.226	Tukey	0.83

T2	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G5	0.00	0.61	176	0.00	0.996	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G2	-0.98	0.61	176	-1.61	0.108	Tukey	0.59
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G3	-1.09	0.61	176	-1.78	0.076	Tukey	0.48
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G4	-0.28	0.61	176	-0.46	0.645	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G5	-1.02	0.61	176	-1.67	0.096	Tukey	0.55
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G3	-0.10	0.61	176	-0.17	0.865	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G4	0.70	0.61	176	1.15	0.250	Tukey	0.86
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G5	-0.04	0.61	176	-0.06	0.954	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G4	0.80	0.61	176	1.32	0.187	Tukey	0.77
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G5	0.07	0.61	176	0.11	0.910	Tukey	1.00
T2	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G4	G5	-0.74	0.61	176	-1.21	0.227	Tukey	0.83
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G1	0.12	0.65	176	0.19	0.850	Tukey	1.00
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G2	-0.37	0.65	176	-0.57	0.567	Tukey	0.99
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G3	-0.66	0.65	176	-1.01	0.314	Tukey	0.91
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G4	-0.88	0.65	176	-1.35	0.180	Tukey	0.76
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G5	-0.19	0.65	176	-0.30	0.766	Tukey	1.00
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G2	-0.50	0.65	176	-0.76	0.447	Tukey	0.97
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G3	-0.78	0.65	176	-1.20	0.232	Tukey	0.84
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G4	-1.00	0.65	176	-1.54	0.126	Tukey	0.64
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G5	-0.32	0.65	176	-0.49	0.627	Tukey	1.00
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G3	-0.28	0.65	176	-0.44	0.663	Tukey	1.00
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G4	-0.50	0.65	176	-0.77	0.440	Tukey	0.97
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G5	0.18	0.65	176	0.28	0.784	Tukey	1.00
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G4	-0.22	0.65	176	-0.34	0.737	Tukey	1.00
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G5	0.46	0.65	176	0.71	0.478	Tukey	0.98
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G4	G5	0.68	0.65	176	1.05	0.296	Tukey	0.90
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G1	-0.11	0.65	176	-0.17	0.866	Tukey	1.00
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G2	0.08	0.65	176	0.12	0.905	Tukey	1.00
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G3	0.68	0.65	176	1.05	0.295	Tukey	0.90
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G4	-0.46	0.65	176	-0.71	0.478	Tukey	0.98
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G5	-0.05	0.65	176	-0.07	0.942	Tukey	1.00
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G2	0.19	0.65	176	0.29	0.773	Tukey	1.00
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G3	0.79	0.65	176	1.22	0.225	Tukey	0.83
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G4	-0.35	0.65	176	-0.54	0.588	Tukey	0.99
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G5	0.06	0.65	176	0.10	0.924	Tukey	1.00
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G3	0.61	0.65	176	0.93	0.354	Tukey	0.94
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G4	-0.54	0.65	176	-0.83	0.407	Tukey	0.96
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G5	-0.13	0.65	176	-0.19	0.847	Tukey	1.00
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G4	-1.15	0.65	176	-1.76	0.080	Tukey	0.49
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G5	-0.73	0.65	176	-1.12	0.263	Tukey	0.87
T3	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G4	G5	0.42	0.65	176	0.64	0.524	Tukey	0.99
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G1	0.46	0.58	176	0.79	0.432	Tukey	0.97
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G2	1.09	0.58	176	1.87	0.063	Tukey	0.43

T3	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G3	0.72	0.58	176	1.23	0.219	Tukey	0.82
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G4	0.32	0.58	176	0.56	0.578	Tukey	0.99
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G5	1.07	0.58	176	1.84	0.067	Tukey	0.44
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G2	0.63	0.58	176	1.08	0.281	Tukey	0.89
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G3	0.26	0.58	176	0.45	0.655	Tukey	1.00
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G4	-0.13	0.58	176	-0.23	0.819	Tukey	1.00
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G5	0.62	0.58	176	1.06	0.292	Tukey	0.90
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G3	-0.37	0.58	176	-0.63	0.527	Tukey	0.99
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G4	-0.76	0.58	176	-1.31	0.191	Tukey	0.78
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G5	-0.02	0.58	176	-0.03	0.980	Tukey	1.00
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G4	-0.40	0.58	176	-0.68	0.499	Tukey	0.98
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G5	0.35	0.58	176	0.61	0.544	Tukey	0.99
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G4	G5	0.75	0.58	176	1.29	0.200	Tukey	0.79
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G1	1.09	0.58	176	1.86	0.064	Tukey	0.43
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G2	0.77	0.58	176	1.33	0.186	Tukey	0.77
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G3	-0.35	0.58	176	-0.60	0.550	Tukey	0.99
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G4	0.50	0.58	176	0.86	0.391	Tukey	0.96
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G5	-0.23	0.58	176	-0.39	0.700	Tukey	1.00
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G2	-0.31	0.58	176	-0.54	0.591	Tukey	0.99
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G3	-1.44	0.58	176	-2.46	0.015	Tukey	0.14
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G4	-0.59	0.58	176	-1.01	0.316	Tukey	0.92
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G5	-1.31	0.58	176	-2.25	0.026	Tukey	0.22
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G3	-1.12	0.58	176	-1.93	0.056	Tukey	0.39
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G4	-0.27	0.58	176	-0.47	0.641	Tukey	1.00
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G5	-1.00	0.58	176	-1.71	0.088	Tukey	0.53
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G4	0.85	0.58	176	1.46	0.147	Tukey	0.69
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G5	0.12	0.58	176	0.21	0.832	Tukey	1.00
T3	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G4	G5	-0.73	0.58	176	-1.25	0.215	Tukey	0.81
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G1	0.30	0.69	176	0.44	0.660	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G2	-0.74	0.69	176	-1.08	0.281	Tukey	0.89
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G3	-0.30	0.69	176	-0.44	0.662	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G4	-0.03	0.69	176	-0.04	0.968	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G5	0.17	0.69	176	0.24	0.809	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G2	-1.04	0.69	176	-1.52	0.130	Tukey	0.65
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G3	-0.60	0.69	176	-0.88	0.381	Tukey	0.95
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G4	-0.33	0.69	176	-0.48	0.632	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G5	-0.14	0.69	176	-0.20	0.843	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G3	0.44	0.69	176	0.64	0.522	Tukey	0.99
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G4	0.71	0.69	176	1.04	0.300	Tukey	0.90
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G5	0.91	0.69	176	1.32	0.188	Tukey	0.77
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G4	0.27	0.69	176	0.40	0.691	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G5	0.47	0.69	176	0.68	0.497	Tukey	0.98
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G4	G5	0.19	0.69	176	0.28	0.778	Tukey	1.00

T4	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G1	-0.78	0.69	176	-1.14	0.256	Tukey	0.86
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G2	-0.47	0.69	176	-0.69	0.493	Tukey	0.98
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G3	0.01	0.69	176	0.01	0.993	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G4	-0.35	0.69	176	-0.51	0.608	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G5	0.09	0.69	176	0.13	0.895	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G2	0.31	0.69	176	0.45	0.652	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G3	0.79	0.69	176	1.15	0.253	Tukey	0.86
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G4	0.43	0.69	176	0.63	0.533	Tukey	0.99
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G5	0.87	0.69	176	1.27	0.205	Tukey	0.80
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G3	0.48	0.69	176	0.70	0.488	Tukey	0.98
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G4	0.12	0.69	176	0.17	0.862	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G5	0.56	0.69	176	0.82	0.414	Tukey	0.96
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G4	-0.36	0.69	176	-0.52	0.602	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G5	0.09	0.69	176	0.12	0.902	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G4	G5	0.44	0.69	176	0.65	0.519	Tukey	0.99
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G1	0.00	0.61	176	-0.01	0.995	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G2	0.12	0.61	176	0.19	0.850	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G3	0.03	0.61	176	0.05	0.964	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G4	-0.09	0.61	176	-0.15	0.879	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G5	0.31	0.61	176	0.50	0.618	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G2	0.12	0.61	176	0.20	0.845	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G3	0.03	0.61	176	0.05	0.959	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G4	-0.09	0.61	176	-0.15	0.884	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G5	0.31	0.61	176	0.51	0.614	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G3	-0.09	0.61	176	-0.14	0.886	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G4	-0.21	0.61	176	-0.34	0.732	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G5	0.19	0.61	176	0.31	0.757	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G4	-0.12	0.61	176	-0.20	0.843	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G5	0.28	0.61	176	0.45	0.651	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G4	G5	0.40	0.61	176	0.65	0.515	Tukey	0.99
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G1	0.71	0.61	176	1.15	0.252	Tukey	0.86
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G2	0.24	0.61	176	0.39	0.698	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G3	0.04	0.61	176	0.06	0.953	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G4	0.53	0.61	176	0.87	0.386	Tukey	0.95
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G5	0.05	0.61	176	0.09	0.932	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G2	-0.47	0.61	176	-0.76	0.448	Tukey	0.97
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G3	-0.67	0.61	176	-1.09	0.277	Tukey	0.88
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G4	-0.17	0.61	176	-0.28	0.779	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G5	-0.65	0.61	176	-1.06	0.289	Tukey	0.90
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G3	-0.20	0.61	176	-0.33	0.742	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G4	0.29	0.61	176	0.48	0.632	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G5	-0.19	0.61	176	-0.30	0.762	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G4	0.50	0.61	176	0.81	0.419	Tukey	0.97

T4	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G5	0.02	0.61	176	0.03	0.979	Tukey	1.00
T4	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G4	G5	-0.48	0.61	176	-0.78	0.435	Tukey	0.97
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G1	1.29	0.74	176	1.75	0.082	Tukey	0.50
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G2	1.06	0.74	176	1.43	0.154	Tukey	0.71
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G3	0.67	0.74	176	0.91	0.364	Tukey	0.94
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G4	0.53	0.74	176	0.72	0.473	Tukey	0.98
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G5	1.00	0.74	176	1.36	0.176	Tukey	0.75
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G2	-0.23	0.74	176	-0.32	0.752	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G3	-0.62	0.74	176	-0.84	0.403	Tukey	0.96
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G4	-0.76	0.74	176	-1.03	0.305	Tukey	0.91
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G5	-0.29	0.74	176	-0.39	0.697	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G3	-0.39	0.74	176	-0.52	0.602	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G4	-0.53	0.74	176	-0.71	0.477	Tukey	0.98
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G5	-0.06	0.74	176	-0.07	0.941	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G4	-0.14	0.74	176	-0.19	0.850	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G5	0.33	0.74	176	0.45	0.654	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G4	G5	0.47	0.74	176	0.64	0.524	Tukey	0.99
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G1	0.03	0.74	176	0.04	0.970	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G2	1.21	0.74	176	1.64	0.103	Tukey	0.57
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G3	0.75	0.74	176	1.01	0.313	Tukey	0.91
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G4	-0.01	0.74	176	-0.01	0.991	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G5	0.43	0.74	176	0.58	0.563	Tukey	0.99
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G2	1.19	0.74	176	1.60	0.111	Tukey	0.60
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G3	0.72	0.74	176	0.97	0.331	Tukey	0.93
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G4	-0.04	0.74	176	-0.05	0.961	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G5	0.40	0.74	176	0.54	0.589	Tukey	0.99
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G3	-0.47	0.74	176	-0.63	0.530	Tukey	0.99
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G4	-1.22	0.74	176	-1.65	0.100	Tukey	0.57
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G5	-0.79	0.74	176	-1.06	0.290	Tukey	0.90
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G4	-0.76	0.74	176	-1.02	0.308	Tukey	0.91
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G5	-0.32	0.74	176	-0.43	0.666	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G4	G5	0.44	0.74	176	0.59	0.556	Tukey	0.99
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G1	0.44	0.66	176	0.66	0.512	Tukey	0.99
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G2	0.18	0.66	176	0.27	0.790	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G3	0.07	0.66	176	0.10	0.919	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G4	0.37	0.66	176	0.56	0.578	Tukey	0.99
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G5	0.79	0.66	176	1.19	0.235	Tukey	0.84
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G2	-0.26	0.66	176	-0.39	0.696	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G3	-0.37	0.66	176	-0.56	0.579	Tukey	0.99
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G4	-0.07	0.66	176	-0.10	0.920	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G5	0.35	0.66	176	0.53	0.595	Tukey	0.99
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G3	-0.11	0.66	176	-0.16	0.869	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G4	0.19	0.66	176	0.29	0.772	Tukey	1.00

T5	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G5	0.61	0.66	176	0.92	0.357	Tukey	0.94
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G4	0.30	0.66	176	0.46	0.649	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G5	0.72	0.66	176	1.09	0.278	Tukey	0.89
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G4	G5	0.42	0.66	176	0.63	0.527	Tukey	0.99
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G1	0.19	0.66	176	0.28	0.780	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G2	-0.19	0.66	176	-0.28	0.780	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G3	-0.51	0.66	176	-0.77	0.443	Tukey	0.97
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G4	-0.32	0.66	176	-0.48	0.632	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G5	-0.52	0.66	176	-0.78	0.436	Tukey	0.97
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G2	-0.37	0.66	176	-0.56	0.576	Tukey	0.99
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G3	-0.69	0.66	176	-1.05	0.296	Tukey	0.90
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G4	-0.50	0.66	176	-0.76	0.448	Tukey	0.97
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G5	-0.70	0.66	176	-1.06	0.290	Tukey	0.90
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G3	-0.32	0.66	176	-0.49	0.626	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G4	-0.13	0.66	176	-0.20	0.841	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G5	-0.33	0.66	176	-0.50	0.617	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G4	0.19	0.66	176	0.29	0.774	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G5	-0.01	0.66	176	-0.01	0.990	Tukey	1.00
T5	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G4	G5	-0.20	0.66	176	-0.30	0.765	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G1	0.51	1.14	176	0.45	0.656	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G2	0.91	1.14	176	0.80	0.423	Tukey	0.97
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G3	0.63	1.14	176	0.55	0.583	Tukey	0.99
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G4	-0.19	1.14	176	-0.17	0.868	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G5	0.87	1.14	176	0.76	0.448	Tukey	0.97
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G2	0.41	1.14	176	0.36	0.722	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G3	0.12	1.14	176	0.10	0.918	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G4	-0.70	1.14	176	-0.61	0.541	Tukey	0.99
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G5	0.36	1.14	176	0.32	0.753	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G3	-0.29	1.14	176	-0.25	0.801	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G4	-1.10	1.14	176	-0.97	0.334	Tukey	0.93
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G5	-0.05	1.14	176	-0.04	0.967	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G4	-0.82	1.14	176	-0.72	0.475	Tukey	0.98
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G5	0.24	1.14	176	0.21	0.833	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G4	G5	1.06	1.14	176	0.93	0.355	Tukey	0.94
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G1	0.35	1.14	176	0.30	0.762	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G2	1.35	1.14	176	1.18	0.238	Tukey	0.84
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G3	2.21	1.14	176	1.94	0.054	Tukey	0.38
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G4	0.94	1.14	176	0.83	0.408	Tukey	0.96
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G5	1.77	1.14	176	1.56	0.122	Tukey	0.63
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G2	1.00	1.14	176	0.88	0.380	Tukey	0.95
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G3	1.86	1.14	176	1.63	0.104	Tukey	0.58
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G4	0.60	1.14	176	0.53	0.600	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G5	1.42	1.14	176	1.25	0.212	Tukey	0.81

T6	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G3	0.86	1.14	176	0.75	0.452	Tukey	0.97
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G4	-0.40	1.14	176	-0.35	0.723	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G5	0.42	1.14	176	0.37	0.711	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G4	-1.26	1.14	176	-1.11	0.269	Tukey	0.88
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G5	-0.44	1.14	176	-0.38	0.702	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G4	G5	0.83	1.14	176	0.73	0.469	Tukey	0.98
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G1	0.75	1.02	176	0.73	0.465	Tukey	0.98
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G2	1.49	1.02	176	1.47	0.145	Tukey	0.69
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G3	1.40	1.02	176	1.38	0.170	Tukey	0.74
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G4	1.60	1.02	176	1.57	0.119	Tukey	0.62
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G5	1.42	1.02	176	1.39	0.166	Tukey	0.73
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G2	0.75	1.02	176	0.73	0.464	Tukey	0.98
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G3	0.66	1.02	176	0.64	0.520	Tukey	0.99
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G4	0.85	1.02	176	0.84	0.405	Tukey	0.96
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G5	0.67	1.02	176	0.66	0.511	Tukey	0.99
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G3	-0.09	1.02	176	-0.09	0.929	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G4	0.10	1.02	176	0.10	0.919	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G5	-0.08	1.02	176	-0.08	0.939	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G4	0.19	1.02	176	0.19	0.849	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G5	0.01	1.02	176	0.01	0.989	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G4	G5	-0.18	1.02	176	-0.18	0.859	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G1	0.92	1.02	176	0.91	0.366	Tukey	0.94
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G2	1.28	1.02	176	1.25	0.212	Tukey	0.81
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G3	0.62	1.02	176	0.60	0.546	Tukey	0.99
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G4	1.43	1.02	176	1.41	0.161	Tukey	0.72
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G5	0.31	1.02	176	0.31	0.758	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G2	0.35	1.02	176	0.35	0.729	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G3	-0.31	1.02	176	-0.30	0.764	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G4	0.51	1.02	176	0.50	0.617	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G5	-0.61	1.02	176	-0.60	0.551	Tukey	0.99
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G3	-0.66	1.02	176	-0.65	0.518	Tukey	0.99
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G4	0.16	1.02	176	0.15	0.878	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G5	-0.96	1.02	176	-0.94	0.346	Tukey	0.93
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G4	0.82	1.02	176	0.80	0.423	Tukey	0.97
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G5	-0.30	1.02	176	-0.30	0.768	Tukey	1.00
T6	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G4	G5	-1.12	1.02	176	-1.10	0.274	Tukey	0.88
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G1	1.10	0.96	176	1.15	0.253	Tukey	0.86
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G2	0.04	0.96	176	0.04	0.970	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G3	-2.00	0.96	176	-2.10	0.037	Tukey	0.29
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G4	-1.01	0.96	176	-1.06	0.292	Tukey	0.90
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G5	0.64	0.96	176	0.67	0.504	Tukey	0.99
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G2	-1.06	0.96	176	-1.11	0.269	Tukey	0.88
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G3	-3.10	0.96	176	-3.24	0.001	Tukey	0.02

T7	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G4	-2.11	0.96	176	-2.20	0.029	Tukey	0.24
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G5	-0.46	0.96	176	-0.48	0.634	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G3	-2.04	0.96	176	-2.13	0.034	Tukey	0.27
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G4	-1.05	0.96	176	-1.09	0.275	Tukey	0.88
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G5	0.60	0.96	176	0.63	0.528	Tukey	0.99
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G4	0.99	0.96	176	1.04	0.299	Tukey	0.90
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G5	2.64	0.96	176	2.77	0.006	Tukey	0.07
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G4	G5	1.65	0.96	176	1.73	0.086	Tukey	0.52
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G1	0.45	0.96	176	0.48	0.635	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G2	2.25	0.96	176	2.35	0.020	Tukey	0.18
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G3	0.27	0.96	176	0.29	0.775	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G4	-0.34	0.96	176	-0.36	0.721	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G5	0.87	0.96	176	0.91	0.362	Tukey	0.94
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G2	1.79	0.96	176	1.87	0.063	Tukey	0.42
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G3	-0.18	0.96	176	-0.19	0.850	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G4	-0.80	0.96	176	-0.83	0.405	Tukey	0.96
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G5	0.42	0.96	176	0.44	0.662	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G3	-1.97	0.96	176	-2.06	0.041	Tukey	0.31
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G4	-2.59	0.96	176	-2.71	0.007	Tukey	0.08
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G5	-1.37	0.96	176	-1.44	0.153	Tukey	0.71
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G4	-0.62	0.96	176	-0.64	0.520	Tukey	0.99
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G5	0.60	0.96	176	0.63	0.531	Tukey	0.99
T7	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G4	G5	1.22	0.96	176	1.27	0.205	Tukey	0.80
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G1	1.33	0.85	176	1.56	0.121	Tukey	0.63
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G2	1.21	0.85	176	1.41	0.159	Tukey	0.72
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G3	0.67	0.85	176	0.78	0.434	Tukey	0.97
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G4	0.07	0.85	176	0.09	0.931	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G5	1.59	0.85	176	1.86	0.065	Tukey	0.43
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G2	-0.12	0.85	176	-0.14	0.886	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G3	-0.66	0.85	176	-0.77	0.440	Tukey	0.97
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G4	-1.26	0.85	176	-1.47	0.143	Tukey	0.68
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G5	0.26	0.85	176	0.30	0.766	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G3	-0.54	0.85	176	-0.63	0.530	Tukey	0.99
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G4	-1.13	0.85	176	-1.33	0.186	Tukey	0.77
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G5	0.38	0.85	176	0.44	0.659	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G4	-0.60	0.85	176	-0.70	0.486	Tukey	0.98
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G5	0.92	0.85	176	1.07	0.285	Tukey	0.89
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G4	G5	1.51	0.85	176	1.77	0.078	Tukey	0.49
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G1	0.34	0.85	176	0.40	0.688	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G2	0.27	0.85	176	0.31	0.755	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G3	0.15	0.85	176	0.17	0.865	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G4	0.36	0.85	176	0.42	0.674	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G5	0.30	0.85	176	0.35	0.728	Tukey	1.00

T7	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G2	-0.08	0.85	176	-0.09	0.928	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G3	-0.20	0.85	176	-0.23	0.817	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G4	0.02	0.85	176	0.02	0.986	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G5	-0.05	0.85	176	-0.05	0.957	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G3	-0.12	0.85	176	-0.14	0.887	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G4	0.09	0.85	176	0.11	0.914	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G5	0.03	0.85	176	0.04	0.972	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G4	0.21	0.85	176	0.25	0.803	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G5	0.15	0.85	176	0.18	0.859	Tukey	1.00
T7	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G4	G5	-0.06	0.85	176	-0.07	0.942	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G1	0.25	1.09	176	0.23	0.817	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G2	-0.74	1.09	176	-0.68	0.497	Tukey	0.98
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G3	-1.17	1.09	176	-1.08	0.282	Tukey	0.89
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G4	0.27	1.09	176	0.24	0.808	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G5	-0.31	1.09	176	-0.29	0.773	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G2	-0.99	1.09	176	-0.91	0.363	Tukey	0.94
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G3	-1.43	1.09	176	-1.31	0.192	Tukey	0.78
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G4	0.01	1.09	176	0.01	0.991	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G5	-0.57	1.09	176	-0.52	0.603	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G3	-0.43	1.09	176	-0.40	0.691	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G4	1.01	1.09	176	0.92	0.357	Tukey	0.94
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G5	0.43	1.09	176	0.39	0.696	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G4	1.44	1.09	176	1.32	0.188	Tukey	0.77
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G5	0.86	1.09	176	0.79	0.431	Tukey	0.97
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G4	G5	-0.58	1.09	176	-0.53	0.595	Tukey	0.99
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G1	1.02	1.09	176	0.94	0.349	Tukey	0.94
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G2	2.45	1.09	176	2.25	0.026	Tukey	0.22
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G3	2.77	1.09	176	2.54	0.012	Tukey	0.12
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G4	0.91	1.09	176	0.84	0.403	Tukey	0.96
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G5	1.10	1.09	176	1.01	0.316	Tukey	0.92
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G2	1.43	1.09	176	1.31	0.192	Tukey	0.78
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G3	1.74	1.09	176	1.60	0.111	Tukey	0.60
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G4	-0.11	1.09	176	-0.10	0.920	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G5	0.07	1.09	176	0.07	0.947	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G3	0.32	1.09	176	0.29	0.770	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G4	-1.53	1.09	176	-1.41	0.161	Tukey	0.72
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G5	-1.35	1.09	176	-1.24	0.216	Tukey	0.82
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G4	-1.85	1.09	176	-1.70	0.090	Tukey	0.53
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G5	-1.67	1.09	176	-1.54	0.126	Tukey	0.64
T8	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G4	G5	0.18	1.09	176	0.17	0.868	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G1	-0.26	0.97	176	-0.27	0.788	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G2	-0.21	0.97	176	-0.22	0.829	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G3	-0.37	0.97	176	-0.38	0.701	Tukey	1.00

T8	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G4	-1.65	0.97	176	-1.70	0.092	Tukey	0.54
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G5	-0.27	0.97	176	-0.28	0.782	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G2	0.05	0.97	176	0.05	0.958	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G3	-0.11	0.97	176	-0.11	0.909	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G4	-1.39	0.97	176	-1.43	0.156	Tukey	0.71
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G5	-0.01	0.97	176	-0.01	0.994	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G3	-0.16	0.97	176	-0.17	0.867	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G4	-1.44	0.97	176	-1.48	0.141	Tukey	0.68
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G5	-0.06	0.97	176	-0.06	0.952	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G4	-1.28	0.97	176	-1.31	0.191	Tukey	0.78
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G5	0.10	0.97	176	0.11	0.915	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G4	G5	1.38	0.97	176	1.42	0.158	Tukey	0.72
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G1	-0.18	0.97	176	-0.18	0.854	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G2	-0.88	0.97	176	-0.90	0.370	Tukey	0.95
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G3	0.05	0.97	176	0.05	0.958	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G4	-0.55	0.97	176	-0.56	0.575	Tukey	0.99
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G5	-0.83	0.97	176	-0.85	0.394	Tukey	0.96
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G2	-0.70	0.97	176	-0.72	0.475	Tukey	0.98
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G3	0.23	0.97	176	0.24	0.813	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G4	-0.37	0.97	176	-0.38	0.706	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G5	-0.65	0.97	176	-0.67	0.503	Tukey	0.98
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G3	0.93	0.97	176	0.95	0.342	Tukey	0.93
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G4	0.33	0.97	176	0.34	0.736	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G5	0.04	0.97	176	0.04	0.964	Tukey	1.00
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G4	-0.60	0.97	176	-0.61	0.540	Tukey	0.99
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G5	-0.88	0.97	176	-0.91	0.365	Tukey	0.94
T8	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G4	G5	-0.29	0.97	176	-0.29	0.770	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G1	0.21	1.00	176	0.21	0.836	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G2	-0.12	1.00	176	-0.12	0.903	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G3	-0.29	1.00	176	-0.29	0.774	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G4	-0.66	1.00	176	-0.66	0.511	Tukey	0.99
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G0	G5	-0.23	1.00	176	-0.23	0.821	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G2	-0.33	1.00	176	-0.33	0.742	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G3	-0.50	1.00	176	-0.50	0.621	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G4	-0.87	1.00	176	-0.87	0.387	Tukey	0.95
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G1	G5	-0.43	1.00	176	-0.43	0.664	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G3	-0.17	1.00	176	-0.17	0.868	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G4	-0.54	1.00	176	-0.54	0.591	Tukey	0.99
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G2	G5	-0.11	1.00	176	-0.11	0.916	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G4	-0.37	1.00	176	-0.37	0.711	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G3	G5	0.06	1.00	176	0.06	0.952	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu m	G4	G5	0.43	1.00	176	0.43	0.666	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G1	0.95	1.00	176	0.95	0.346	Tukey	0.93

T9	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G2	2.66	1.00	176	2.66	0.009	Tukey	0.09
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G3	1.36	1.00	176	1.36	0.175	Tukey	0.75
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G4	0.48	1.00	176	0.48	0.635	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G0	G5	0.21	1.00	176	0.21	0.836	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G2	1.71	1.00	176	1.71	0.089	Tukey	0.53
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G3	0.42	1.00	176	0.42	0.677	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G4	-0.47	1.00	176	-0.47	0.640	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G1	G5	-0.74	1.00	176	-0.74	0.462	Tukey	0.98
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G3	-1.30	1.00	176	-1.30	0.197	Tukey	0.79
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G4	-2.18	1.00	176	-2.18	0.031	Tukey	0.25
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G2	G5	-2.45	1.00	176	-2.45	0.015	Tukey	0.15
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G4	-0.89	1.00	176	-0.89	0.377	Tukey	0.95
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G3	G5	-1.15	1.00	176	-1.15	0.250	Tukey	0.86
T9	3	AgeCat*Trt*Glu	AgeCat Old Glu p	G4	G5	-0.27	1.00	176	-0.27	0.789	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G1	-0.06	0.89	176	-0.06	0.949	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G2	-0.12	0.89	176	-0.14	0.892	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G3	-0.31	0.89	176	-0.34	0.731	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G4	-0.48	0.89	176	-0.54	0.591	Tukey	0.99
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G0	G5	0.61	0.89	176	0.68	0.498	Tukey	0.98
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G2	-0.06	0.89	176	-0.07	0.943	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G3	-0.25	0.89	176	-0.28	0.780	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G4	-0.42	0.89	176	-0.47	0.636	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G1	G5	0.67	0.89	176	0.74	0.458	Tukey	0.98
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G3	-0.19	0.89	176	-0.21	0.835	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G4	-0.36	0.89	176	-0.40	0.688	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G2	G5	0.73	0.89	176	0.82	0.416	Tukey	0.96
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G4	-0.17	0.89	176	-0.19	0.847	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G3	G5	0.92	0.89	176	1.02	0.307	Tukey	0.91
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu m	G4	G5	1.09	0.89	176	1.22	0.225	Tukey	0.83
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G1	-0.94	0.89	176	-1.06	0.293	Tukey	0.90
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G2	-0.91	0.89	176	-1.02	0.308	Tukey	0.91
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G3	-0.59	0.89	176	-0.66	0.512	Tukey	0.99
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G4	-1.19	0.89	176	-1.33	0.185	Tukey	0.77
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G0	G5	-0.72	0.89	176	-0.80	0.424	Tukey	0.97
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G2	0.03	0.89	176	0.03	0.973	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G3	0.36	0.89	176	0.40	0.690	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G4	-0.25	0.89	176	-0.28	0.783	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G1	G5	0.23	0.89	176	0.25	0.800	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G3	0.33	0.89	176	0.37	0.715	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G4	-0.28	0.89	176	-0.31	0.758	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G2	G5	0.20	0.89	176	0.22	0.826	Tukey	1.00
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G4	-0.60	0.89	176	-0.67	0.501	Tukey	0.98
T9	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G3	G5	-0.13	0.89	176	-0.14	0.885	Tukey	1.00

T9	3	AgeCat*Trt*Glu	AgeCat Young Glu p	G4	G5	0.47	0.89	176	0.53	0.597	Tukey	0.99

TEST 5: gives comparisons of glucose (m) versus (p) for each gene, age and treatment:

Gene	St No	Effect	Slice	Glu	Glu	Estimate	StdErr	DF	tValue	Raw_p	fdr_p
T1	4	AgeCat*Trt*Glu	AgeCat Old Trt G0	M	p	-0.70	0.63	176	-1.11	0.27	0.77
T1	4	AgeCat*Trt*Glu	AgeCat Old Trt G1	M	p	-0.48	0.63	176	-0.76	0.45	0.77
T1	4	AgeCat*Trt*Glu	AgeCat Old Trt G2	M	p	-0.49	0.63	176	-0.77	0.44	0.77
T1	4	AgeCat*Trt*Glu	AgeCat Old Trt G3	M	p	-0.05	0.63	176	-0.08	0.94	0.94
T1	4	AgeCat*Trt*Glu	AgeCat Old Trt G4	M	p	-0.39	0.63	176	-0.61	0.54	0.81
T1	4	AgeCat*Trt*Glu	AgeCat Old Trt G5	M	p	-0.85	0.63	176	-1.34	0.18	0.77
T1	4	AgeCat*Trt*Glu	AgeCat Young Trt G0	M	p	-0.26	0.56	176	-0.47	0.64	0.86
T1	4	AgeCat*Trt*Glu	AgeCat Young Trt G1	M	p	0.87	0.56	176	1.54	0.13	0.77
T1	4	AgeCat*Trt*Glu	AgeCat Young Trt G2	M	p	-0.45	0.56	176	-0.80	0.43	0.77
T1	4	AgeCat*Trt*Glu	AgeCat Young Trt G3	M	p	-0.07	0.56	176	-0.12	0.90	0.94
T1	4	AgeCat*Trt*Glu	AgeCat Young Trt G4	M	p	0.20	0.56	176	0.35	0.73	0.87
T1	4	AgeCat*Trt*Glu	AgeCat Young Trt G5	M	p	-0.58	0.56	176	-1.02	0.31	0.77
T10	4	AgeCat*Trt*Glu	AgeCat Old Trt G0	M	p	-1.97	0.84	176	-2.34	0.02	0.20
T10	4	AgeCat*Trt*Glu	AgeCat Old Trt G1	M	p	-1.50	0.84	176	-1.78	0.08	0.30
T10	4	AgeCat*Trt*Glu	AgeCat Old Trt G2	M	p	1.09	0.84	176	1.30	0.20	0.34
T10	4	AgeCat*Trt*Glu	AgeCat Old Trt G3	M	p	0.12	0.84	176	0.15	0.88	0.88
T10	4	AgeCat*Trt*Glu	AgeCat Old Trt G4	M	p	-1.11	0.84	176	-1.32	0.19	0.34
T10	4	AgeCat*Trt*Glu	AgeCat Old Trt G5	M	p	-0.21	0.84	176	-0.25	0.81	0.88
T10	4	AgeCat*Trt*Glu	AgeCat Young Trt G0	M	p	-1.06	0.75	176	-1.41	0.16	0.34
T10	4	AgeCat*Trt*Glu	AgeCat Young Trt G1	M	p	-0.58	0.75	176	-0.77	0.44	0.66
T10	4	AgeCat*Trt*Glu	AgeCat Young Trt G2	M	p	-0.49	0.75	176	-0.65	0.52	0.69
T10	4	AgeCat*Trt*Glu	AgeCat Young Trt G3	M	p	-0.98	0.75	176	-1.31	0.19	0.34
T10	4	AgeCat*Trt*Glu	AgeCat Young Trt G4	M	p	-0.12	0.75	176	-0.15	0.88	0.88
T10	4	AgeCat*Trt*Glu	AgeCat Young Trt G5	M	p	-1.60	0.75	176	-2.14	0.03	0.20
T2	4	AgeCat*Trt*Glu	AgeCat Old Trt G0	M	p	-1.42	0.68	176	-2.09	0.04	0.19
T2	4	AgeCat*Trt*Glu	AgeCat Old Trt G1	M	p	-1.36	0.68	176	-2.00	0.05	0.19
T2	4	AgeCat*Trt*Glu	AgeCat Old Trt G2	M	p	-0.72	0.68	176	-1.06	0.29	0.49
T2	4	AgeCat*Trt*Glu	AgeCat Old Trt G3	M	p	-0.20	0.68	176	-0.29	0.77	0.77
T2	4	AgeCat*Trt*Glu	AgeCat Old Trt G4	M	p	-0.38	0.68	176	-0.56	0.58	0.77
T2	4	AgeCat*Trt*Glu	AgeCat Old Trt G5	M	p	-1.16	0.68	176	-1.70	0.09	0.27
T2	4	AgeCat*Trt*Glu	AgeCat Young Trt G0	M	p	-0.19	0.61	176	-0.31	0.76	0.77
T2	4	AgeCat*Trt*Glu	AgeCat Young Trt G1	M	p	0.72	0.61	176	1.18	0.24	0.48
T2	4	AgeCat*Trt*Glu	AgeCat Young Trt G2	M	p	-1.26	0.61	176	-2.08	0.04	0.19
T2	4	AgeCat*Trt*Glu	AgeCat Young Trt G3	M	p	-0.26	0.61	176	-0.44	0.66	0.77
T2	4	AgeCat*Trt*Glu	AgeCat Young Trt G4	M	p	0.23	0.61	176	0.38	0.70	0.77
T2	4	AgeCat*Trt*Glu	AgeCat Young Trt G5	M	p	-0.84	0.61	176	-1.39	0.17	0.40
T3	4	AgeCat*Trt*Glu	AgeCat Old Trt G0	M	p	-1.18	0.65	176	-1.81	0.07	0.31
T3	4	AgeCat*Trt*Glu	AgeCat Old Trt G1	M	p	-1.41	0.65	176	-2.17	0.03	0.31
T3	4	AgeCat*Trt*Glu	AgeCat Old Trt G2	M	p	-0.73	0.65	176	-1.11	0.27	0.40
T3	4	geCat*Trt*Glu	AgeCat Old Trt G3	M	p	0.16	0.65	176	0.25	0.80	0.87

T3	4	AgeCat*Trt*Glu	AgeCat Old Trt G4	M	p	-0.76	0.65	176	-1.17	0.24	0.40
T3	4	AgeCat*Trt*Glu	AgeCat Old Trt G5	M	p	-1.03	0.65	176	-1.58	0.12	0.31
T3	4	AgeCat*Trt*Glu	AgeCat Young Trt G0	M	p	0.41	0.58	176	0.70	0.48	0.58
T3	4	AgeCat*Trt*Glu	AgeCat Young Trt G1	M	p	1.04	0.58	176	1.78	0.08	0.31
T3	4	AgeCat*Trt*Glu	AgeCat Young Trt G2	M	p	0.09	0.58	176	0.16	0.87	0.87
T3	4	AgeCat*Trt*Glu	AgeCat Young Trt G3	M	p	-0.66	0.58	176	-1.13	0.26	0.40
T3	4	AgeCat*Trt*Glu	AgeCat Young Trt G4	M	p	0.59	0.58	176	1.01	0.32	0.42
T3	4	AgeCat*Trt*Glu	AgeCat Young Trt G5	M	p	-0.89	0.58	176	-1.53	0.13	0.31
T4	4	AgeCat*Trt*Glu	AgeCat Old Trt G0	M	p	-0.23	0.69	176	-0.34	0.73	0.88
T4	4	AgeCat*Trt*Glu	AgeCat Old Trt G1	M	p	-1.32	0.69	176	-1.92	0.06	0.68
T4	4	AgeCat*Trt*Glu	AgeCat Old Trt G2	M	p	0.04	0.69	176	0.05	0.96	0.96
T4	4	AgeCat*Trt*Glu	AgeCat Old Trt G3	M	p	0.07	0.69	176	0.11	0.92	0.96
T4	4	AgeCat*Trt*Glu	AgeCat Old Trt G4	M	p	-0.56	0.69	176	-0.82	0.42	0.88
T4	4	AgeCat*Trt*Glu	AgeCat Old Trt G5	M	p	-0.31	0.69	176	-0.45	0.65	0.88
T4	4	AgeCat*Trt*Glu	AgeCat Young Trt G0	M	p	-0.38	0.61	176	-0.62	0.54	0.88
T4	4	AgeCat*Trt*Glu	AgeCat Young Trt G1	M	p	0.33	0.61	176	0.54	0.59	0.88
T4	4	AgeCat*Trt*Glu	AgeCat Young Trt G2	M	p	-0.26	0.61	176	-0.42	0.68	0.88
T4	4	AgeCat*Trt*Glu	AgeCat Young Trt G3	M	p	-0.37	0.61	176	-0.60	0.55	0.88
T4	4	AgeCat*Trt*Glu	AgeCat Young Trt G4	M	p	0.25	0.61	176	0.40	0.69	0.88
T4	4	AgeCat*Trt*Glu	AgeCat Young Trt G5	M	p	-0.63	0.61	176	-1.03	0.30	0.88
T5	4	AgeCat*Trt*Glu	AgeCat Old Trt G0	M	p	0.07	0.74	176	0.09	0.93	0.93
T5	4	AgeCat*Trt*Glu	AgeCat Old Trt G1	M	p	-1.20	0.74	176	-1.62	0.11	0.93
T5	4	AgeCat*Trt*Glu	AgeCat Old Trt G2	M	p	0.22	0.74	176	0.30	0.77	0.93
T5	4	AgeCat*Trt*Glu	AgeCat Old Trt G3	M	p	0.14	0.74	176	0.19	0.85	0.93
T5	4	AgeCat*Trt*Glu	AgeCat Old Trt G4	M	p	-0.47	0.74	176	-0.64	0.52	0.93
T5	4	AgeCat*Trt*Glu	AgeCat Old Trt G5	M	p	-0.51	0.74	176	-0.69	0.49	0.93
T5	4	AgeCat*Trt*Glu	AgeCat Young Trt G0	M	p	0.46	0.66	176	0.70	0.48	0.93
T5	4	AgeCat*Trt*Glu	AgeCat Young Trt G1	M	p	0.21	0.66	176	0.32	0.75	0.93
T5	4	AgeCat*Trt*Glu	AgeCat Young Trt G2	M	p	0.10	0.66	176	0.16	0.88	0.93
T5	4	AgeCat*Trt*Glu	AgeCat Young Trt G3	M	p	-0.11	0.66	176	-0.17	0.87	0.93
T5	4	AgeCat*Trt*Glu	AgeCat Young Trt G4	M	p	-0.22	0.66	176	-0.34	0.74	0.93
T5	4	AgeCat*Trt*Glu	AgeCat Young Trt G5	M	p	-0.84	0.66	176	-1.27	0.21	0.93
T6	4	AgeCat*Trt*Glu	AgeCat Old Trt G0	M	p	-1.11	1.14	176	-0.97	0.33	0.99
T6	4	AgeCat*Trt*Glu	AgeCat Old Trt G1	M	p	-1.27	1.14	176	-1.12	0.27	0.99
T6	4	AgeCat*Trt*Glu	AgeCat Old Trt G2	M	p	-0.67	1.14	176	-0.59	0.55	0.99
T6	4	AgeCat*Trt*Glu	AgeCat Old Trt G3	M	p	0.47	1.14	176	0.41	0.68	0.99
T6	4	AgeCat*Trt*Glu	AgeCat Old Trt G4	M	p	0.02	1.14	176	0.02	0.98	0.99
T6	4	AgeCat*Trt*Glu	AgeCat Old Trt G5	M	p	-0.20	1.14	176	-0.18	0.86	0.99
T6	4	AgeCat*Trt*Glu	AgeCat Young Trt G0	M	p	0.20	1.02	176	0.20	0.84	0.99
T6	4	AgeCat*Trt*Glu	AgeCat Young Trt G1	M	p	0.38	1.02	176	0.37	0.71	0.99
T6	4	AgeCat*Trt*Glu	AgeCat Young Trt G2	M	p	-0.02	1.02	176	-0.02	0.99	0.99
T6	4	AgeCat*Trt*Glu	AgeCat Young Trt G3	M	p	-0.59	1.02	176	-0.58	0.57	0.99
T6	4	AgeCat*Trt*Glu	AgeCat Young Trt G4	M	p	0.04	1.02	176	0.04	0.97	0.99

T6	4	AgeCat*Trt*Glu	AgeCat Young Trt G5	M	p	-0.90	1.02	176	-0.88	0.38	0.99
T7	4	AgeCat*Trt*Glu	AgeCat Old Trt G0	M	p	-0.96	0.96	176	-1.01	0.31	0.75
T7	4	AgeCat*Trt*Glu	AgeCat Old Trt G1	M	p	-1.61	0.96	176	-1.68	0.09	0.75
T7	4	AgeCat*Trt*Glu	AgeCat Old Trt G2	M	p	1.24	0.96	176	1.30	0.19	0.75
T7	4	AgeCat*Trt*Glu	AgeCat Old Trt G3	M	p	1.31	0.96	176	1.37	0.17	0.75
T7	4	AgeCat*Trt*Glu	AgeCat Old Trt G4	M	p	-0.30	0.96	176	-0.31	0.76	0.82
T7	4	AgeCat*Trt*Glu	AgeCat Old Trt G5	M	p	-0.73	0.96	176	-0.77	0.45	0.76
T7	4	AgeCat*Trt*Glu	AgeCat Young Trt G0	M	p	0.53	0.85	176	0.62	0.54	0.76
T7	4	AgeCat*Trt*Glu	AgeCat Young Trt G1	M	p	-0.46	0.85	176	-0.53	0.59	0.76
T7	4	AgeCat*Trt*Glu	AgeCat Young Trt G2	M	p	-0.41	0.85	176	-0.48	0.63	0.76
T7	4	AgeCat*Trt*Glu	AgeCat Young Trt G3	M	p	0.01	0.85	176	0.01	0.99	0.99
T7	4	AgeCat*Trt*Glu	AgeCat Young Trt G4	M	p	0.82	0.85	176	0.96	0.34	0.75
T7	4	AgeCat*Trt*Glu	AgeCat Young Trt G5	M	p	-0.76	0.85	176	-0.89	0.38	0.75
T8	4	AgeCat*Trt*Glu	AgeCat Old Trt G0	M	p	-1.88	1.09	176	-1.72	0.09	0.52
T8	4	AgeCat*Trt*Glu	AgeCat Old Trt G1	M	p	-1.11	1.09	176	-1.02	0.31	0.54
T8	4	AgeCat*Trt*Glu	AgeCat Old Trt G2	M	p	1.31	1.09	176	1.20	0.23	0.54
T8	4	AgeCat*Trt*Glu	AgeCat Old Trt G3	M	p	2.06	1.09	176	1.90	0.06	0.52
T8	4	AgeCat*Trt*Glu	AgeCat Old Trt G4	M	p	-1.23	1.09	176	-1.13	0.26	0.54
T8	4	AgeCat*Trt*Glu	AgeCat Old Trt G5	M	p	-0.47	1.09	176	-0.43	0.67	0.80
T8	4	AgeCat*Trt*Glu	AgeCat Young Trt G0	M	p	-0.42	0.97	176	-0.43	0.67	0.80
T8	4	AgeCat*Trt*Glu	AgeCat Young Trt G1	M	p	-0.33	0.97	176	-0.34	0.73	0.80
T8	4	AgeCat*Trt*Glu	AgeCat Young Trt G2	M	p	-1.08	0.97	176	-1.11	0.27	0.54
T8	4	AgeCat*Trt*Glu	AgeCat Young Trt G3	M	p	0.01	0.97	176	0.01	0.99	0.99
T8	4	AgeCat*Trt*Glu	AgeCat Young Trt G4	M	p	0.69	0.97	176	0.71	0.48	0.72
T8	4	AgeCat*Trt*Glu	AgeCat Young Trt G5	M	p	-0.98	0.97	176	-1.00	0.32	0.54
T9	4	AgeCat*Trt*Glu	AgeCat Old Trt G0	M	p	-0.77	1.00	176	-0.77	0.44	0.70
T9	4	AgeCat*Trt*Glu	AgeCat Old Trt G1	M	p	-0.04	1.00	176	-0.04	0.97	0.97
T9	4	AgeCat*Trt*Glu	AgeCat Old Trt G2	M	p	2.01	1.00	176	2.00	0.05	0.36
T9	4	AgeCat*Trt*Glu	AgeCat Old Trt G3	M	p	0.88	1.00	176	0.88	0.38	0.70
T9	4	AgeCat*Trt*Glu	AgeCat Old Trt G4	M	p	0.36	1.00	176	0.36	0.72	0.80
T9	4	AgeCat*Trt*Glu	AgeCat Old Trt G5	M	p	-0.34	1.00	176	-0.34	0.74	0.80
T9	4	AgeCat*Trt*Glu	AgeCat Young Trt G0	M	p	-0.37	0.89	176	-0.41	0.68	0.80
T9	4	AgeCat*Trt*Glu	AgeCat Young Trt G1	M	p	-1.26	0.89	176	-1.40	0.16	0.55
T9	4	AgeCat*Trt*Glu	AgeCat Young Trt G2	M	p	-1.16	0.89	176	-1.30	0.20	0.55
T9	4	AgeCat*Trt*Glu	AgeCat Young Trt G3	M	p	-0.65	0.89	176	-0.72	0.47	0.70
T9	4	AgeCat*Trt*Glu	AgeCat Young Trt G4	M	p	-1.08	0.89	176	-1.21	0.23	0.55
T9	4	AgeCat*Trt*Glu	AgeCat Young Trt G5	M	p	-1.69	0.89	176	-1.89	0.06	0.36

TEST 6: gives comparisons of ages for each gene, glucose level and treatment separately:

Gene	StNo	Effect	Slice	AgeCat	Age	Esti	StdErr	DF	tValue	Raw p
T1	5	AgeCat*Trt*Glu	Trt G0 Glu m	Old	Young	-1.98	0.94	39.74	-2.12	0.04
T1	5	AgeCat*Trt*Glu	Trt G0 Glu p	Old	Young	-1.55	0.94	39.74	-1.65	0.11
T1	5	AgeCat*Trt*Glu	Trt G1 Glu m	Old	Young	-1.64	0.94	39.74	-1.76	0.09
T1	5	AgeCat*Trt*Glu	Trt G1 Glu p	Old	Young	-0.30	0.94	39.74	-0.32	0.75
T1	5	AgeCat*Trt*Glu	Trt G2 Glu m	Old	Young	-1.16	0.94	39.74	-1.24	0.22
T1	5	AgeCat*Trt*Glu	Trt G2 Glu p	Old	Young	-1.12	0.94	39.74	-1.20	0.24
T1	5	AgeCat*Trt*Glu	Trt G3 Glu m	Old	Young	-1.47	0.94	39.74	-1.57	0.12
T1	5	AgeCat*Trt*Glu	Trt G3 Glu p	Old	Young	-1.49	0.94	39.74	-1.59	0.12
T1	5	AgeCat*Trt*Glu	Trt G4 Glu m	Old	Young	-0.45	0.94	39.74	-0.48	0.64
T1	5	AgeCat*Trt*Glu	Trt G4 Glu p	Old	Young	0.14	0.94	39.74	0.15	0.88
T1	5	AgeCat*Trt*Glu	Trt G5 Glu m	Old	Young	-1.47	0.94	39.74	-1.57	0.12
T1	5	AgeCat*Trt*Glu	Trt G5 Glu p	Old	Young	-1.20	0.94	39.74	-1.28	0.21
T10	5	AgeCat*Trt*Glu	Trt G0 Glu m	Old	Young	-0.75	1.30	36.36	-0.58	0.57
T10	5	AgeCat*Trt*Glu	Trt G0 Glu p	Old	Young	0.16	1.30	36.36	0.12	0.90
T10	5	AgeCat*Trt*Glu	Trt G1 Glu m	Old	Young	-1.62	1.30	36.36	-1.25	0.22
T10	5	AgeCat*Trt*Glu	Trt G1 Glu p	Old	Young	-0.70	1.30	36.36	-0.54	0.59
T10	5	AgeCat*Trt*Glu	Trt G2 Glu m	Old	Young	-0.06	1.30	36.36	-0.05	0.96
T10	5	AgeCat*Trt*Glu	Trt G2 Glu p	Old	Young	-1.63	1.30	36.36	-1.26	0.22
T10	5	AgeCat*Trt*Glu	Trt G3 Glu m	Old	Young	-0.02	1.30	36.36	-0.01	0.99
T10	5	AgeCat*Trt*Glu	Trt G3 Glu p	Old	Young	-1.12	1.30	36.36	-0.86	0.39
T10	5	AgeCat*Trt*Glu	Trt G4 Glu m	Old	Young	-0.44	1.30	36.36	-0.34	0.74
T10	5	AgeCat*Trt*Glu	Trt G4 Glu p	Old	Young	0.56	1.30	36.36	0.43	0.67
T10	5	AgeCat*Trt*Glu	Trt G5 Glu m	Old	Young	0.13	1.30	36.36	0.10	0.92
T10	5	AgeCat*Trt*Glu	Trt G5 Glu p	Old	Young	-1.26	1.30	36.36	-0.97	0.34
T2	5	AgeCat*Trt*Glu	Trt G0 Glu m	Old	Young	-2.35	0.92	49.81	-2.56	0.01
T2	5	AgeCat*Trt*Glu	Trt G0 Glu p	Old	Young	-1.12	0.92	49.81	-1.21	0.23
T2	5	AgeCat*Trt*Glu	Trt G1 Glu m	Old	Young	-1.55	0.92	49.81	-1.68	0.10
T2	5	AgeCat*Trt*Glu	Trt G1 Glu p	Old	Young	0.53	0.92	49.81	0.58	0.57
T2	5	AgeCat*Trt*Glu	Trt G2 Glu m	Old	Young	-0.77	0.92	49.81	-0.84	0.41
T2	5	AgeCat*Trt*Glu	Trt G2 Glu p	Old	Young	-1.31	0.92	49.81	-1.42	0.16
T2	5	AgeCat*Trt*Glu	Trt G3 Glu m	Old	Young	-1.83	0.92	49.81	-1.99	0.05
T2	5	AgeCat*Trt*Glu	Trt G3 Glu p	Old	Young	-1.90	0.92	49.81	-2.06	0.04
T2	5	AgeCat*Trt*Glu	Trt G4 Glu m	Old	Young	-0.58	0.92	49.81	-0.63	0.53
T2	5	AgeCat*Trt*Glu	Trt G4 Glu p	Old	Young	0.04	0.92	49.81	0.04	0.97
T2	5	AgeCat*Trt*Glu	Trt G5 Glu m	Old	Young	-1.55	0.92	49.81	-1.69	0.10
T2	5	AgeCat*Trt*Glu	Trt G5 Glu p	Old	Young	-1.24	0.92	49.81	-1.35	0.18
T3	5	AgeCat*Trt*Glu	Trt G0 Glu m	Old	Young	-3.12	0.88	49.58	-3.53	0.00
T3	5	AgeCat*Trt*Glu	Trt G0 Glu p	Old	Young	-1.53	0.88	49.58	-1.73	0.09
T3	5	AgeCat*Trt*Glu	Trt G1 Glu m	Old	Young	-2.78	0.88	49.58	-3.15	0.00
T3	5	AgeCat*Trt*Glu	Trt G1 Glu p	Old	Young	-0.33	0.88	49.58	-0.38	0.71

T3	5	AgeCat*Trt*Glu	Trt G2 Glu m	Old	Young	-1.66	0.88	49.58	-1.87	0.07
T3	5	AgeCat*Trt*Glu	Trt G2 Glu p	Old	Young	-0.83	0.88	49.58	-0.94	0.35
T3	5	AgeCat*Trt*Glu	Trt G3 Glu m	Old	Young	-1.74	0.88	49.58	-1.97	0.05
T3	5	AgeCat*Trt*Glu	Trt G3 Glu p	Old	Young	-2.56	0.88	49.58	-2.90	0.01
T3	5	AgeCat*Trt*Glu	Trt G4 Glu m	Old	Young	-1.92	0.88	49.58	-2.17	0.04
T3	5	AgeCat*Trt*Glu	Trt G4 Glu p	Old	Young	-0.57	0.88	49.58	-0.64	0.53
T3	5	AgeCat*Trt*Glu	Trt G5 Glu m	Old	Young	-1.85	0.88	49.58	-2.09	0.04
T3	5	AgeCat*Trt*Glu	Trt G5 Glu p	Old	Young	-1.71	0.88	49.58	-1.93	0.06
T4	5	AgeCat*Trt*Glu	Trt G0 Glu m	Old	Young	-0.64	0.78	94.43	-0.82	0.42
T4	5	AgeCat*Trt*Glu	Trt G0 Glu p	Old	Young	-0.78	0.78	94.43	-1.00	0.32
T4	5	AgeCat*Trt*Glu	Trt G1 Glu m	Old	Young	-0.94	0.78	94.43	-1.21	0.23
T4	5	AgeCat*Trt*Glu	Trt G1 Glu p	Old	Young	0.71	0.78	94.43	0.90	0.37
T4	5	AgeCat*Trt*Glu	Trt G2 Glu m	Old	Young	0.22	0.78	94.43	0.28	0.78
T4	5	AgeCat*Trt*Glu	Trt G2 Glu p	Old	Young	-0.07	0.78	94.43	-0.09	0.93
T4	5	AgeCat*Trt*Glu	Trt G3 Glu m	Old	Young	-0.31	0.78	94.43	-0.40	0.69
T4	5	AgeCat*Trt*Glu	Trt G3 Glu p	Old	Young	-0.75	0.78	94.43	-0.96	0.34
T4	5	AgeCat*Trt*Glu	Trt G4 Glu m	Old	Young	-0.70	0.78	94.43	-0.90	0.37
T4	5	AgeCat*Trt*Glu	Trt G4 Glu p	Old	Young	0.10	0.78	94.43	0.13	0.89
T4	5	AgeCat*Trt*Glu	Trt G5 Glu m	Old	Young	-0.50	0.78	94.43	-0.64	0.53
T4	5	AgeCat*Trt*Glu	Trt G5 Glu p	Old	Young	-0.82	0.78	94.43	-1.05	0.30
T5	5	AgeCat*Trt*Glu	Trt G0 Glu m	Old	Young	1.76	1.62	23.21	1.09	0.29
T5	5	AgeCat*Trt*Glu	Trt G0 Glu p	Old	Young	2.16	1.62	23.21	1.33	0.20
T5	5	AgeCat*Trt*Glu	Trt G1 Glu m	Old	Young	0.91	1.62	23.21	0.56	0.58
T5	5	AgeCat*Trt*Glu	Trt G1 Glu p	Old	Young	2.32	1.62	23.21	1.43	0.17
T5	5	AgeCat*Trt*Glu	Trt G2 Glu m	Old	Young	0.88	1.62	23.21	0.54	0.59
T5	5	AgeCat*Trt*Glu	Trt G2 Glu p	Old	Young	0.76	1.62	23.21	0.47	0.64
T5	5	AgeCat*Trt*Glu	Trt G3 Glu m	Old	Young	1.16	1.62	23.21	0.71	0.48
T5	5	AgeCat*Trt*Glu	Trt G3 Glu p	Old	Young	0.90	1.62	23.21	0.56	0.58
T5	5	AgeCat*Trt*Glu	Trt G4 Glu m	Old	Young	1.60	1.62	23.21	0.99	0.33
T5	5	AgeCat*Trt*Glu	Trt G4 Glu p	Old	Young	1.85	1.62	23.21	1.14	0.27
T5	5	AgeCat*Trt*Glu	Trt G5 Glu m	Old	Young	1.55	1.62	23.21	0.95	0.35
T5	5	AgeCat*Trt*Glu	Trt G5 Glu p	Old	Young	1.22	1.62	23.21	0.75	0.46
T6	5	AgeCat*Trt*Glu	Trt G0 Glu m	Old	Young	-0.99	1.80	34.97	-0.55	0.58
T6	5	AgeCat*Trt*Glu	Trt G0 Glu p	Old	Young	0.32	1.80	34.97	0.18	0.86
T6	5	AgeCat*Trt*Glu	Trt G1 Glu m	Old	Young	-0.75	1.80	34.97	-0.42	0.68
T6	5	AgeCat*Trt*Glu	Trt G1 Glu p	Old	Young	0.89	1.80	34.97	0.50	0.62
T6	5	AgeCat*Trt*Glu	Trt G2 Glu m	Old	Young	-0.41	1.80	34.97	-0.23	0.82
T6	5	AgeCat*Trt*Glu	Trt G2 Glu p	Old	Young	0.24	1.80	34.97	0.14	0.89
T6	5	AgeCat*Trt*Glu	Trt G3 Glu m	Old	Young	-0.22	1.80	34.97	-0.12	0.91
T6	5	AgeCat*Trt*Glu	Trt G3 Glu p	Old	Young	-1.27	1.80	34.97	-0.71	0.48
T6	5	AgeCat*Trt*Glu	Trt G4 Glu m	Old	Young	0.79	1.80	34.97	0.44	0.66
T6	5	AgeCat*Trt*Glu	Trt G4 Glu p	Old	Young	0.81	1.80	34.97	0.45	0.66

T6	5	AgeCat*Trt*Glu	Trt G5 Glu m	Old	Young	-0.44	1.80	34.97	-0.25	0.81
T6	5	AgeCat*Trt*Glu	Trt G5 Glu p	Old	Young	-1.14	1.80	34.97	-0.63	0.53
T7	5	AgeCat*Trt*Glu	Trt G0 Glu m	Old	Young	-2.90	1.22	59.56	-2.37	0.02
T7	5	AgeCat*Trt*Glu	Trt G0 Glu p	Old	Young	-1.40	1.22	59.56	-1.15	0.26
T7	5	AgeCat*Trt*Glu	Trt G1 Glu m	Old	Young	-2.66	1.22	59.56	-2.18	0.03
T7	5	AgeCat*Trt*Glu	Trt G1 Glu p	Old	Young	-1.51	1.22	59.56	-1.24	0.22
T7	5	AgeCat*Trt*Glu	Trt G2 Glu m	Old	Young	-1.72	1.22	59.56	-1.41	0.16
T7	5	AgeCat*Trt*Glu	Trt G2 Glu p	Old	Young	-3.38	1.22	59.56	-2.76	0.01
T7	5	AgeCat*Trt*Glu	Trt G3 Glu m	Old	Young	-0.22	1.22	59.56	-0.18	0.86
T7	5	AgeCat*Trt*Glu	Trt G3 Glu p	Old	Young	-1.53	1.22	59.56	-1.25	0.22
T7	5	AgeCat*Trt*Glu	Trt G4 Glu m	Old	Young	-1.81	1.22	59.56	-1.48	0.14
T7	5	AgeCat*Trt*Glu	Trt G4 Glu p	Old	Young	-0.70	1.22	59.56	-0.57	0.57
T7	5	AgeCat*Trt*Glu	Trt G5 Glu m	Old	Young	-1.95	1.22	59.56	-1.59	0.12
T7	5	AgeCat*Trt*Glu	Trt G5 Glu p	Old	Young	-1.98	1.22	59.56	-1.62	0.11
T8	5	AgeCat*Trt*Glu	Trt G0 Glu m	Old	Young	1.15	1.78	33.00	0.65	0.52
T8	5	AgeCat*Trt*Glu	Trt G0 Glu p	Old	Young	2.61	1.78	33.00	1.47	0.15
T8	5	AgeCat*Trt*Glu	Trt G1 Glu m	Old	Young	0.63	1.78	33.00	0.36	0.72
T8	5	AgeCat*Trt*Glu	Trt G1 Glu p	Old	Young	1.41	1.78	33.00	0.79	0.43
T8	5	AgeCat*Trt*Glu	Trt G2 Glu m	Old	Young	1.68	1.78	33.00	0.94	0.35
T8	5	AgeCat*Trt*Glu	Trt G2 Glu p	Old	Young	-0.71	1.78	33.00	-0.40	0.69
T8	5	AgeCat*Trt*Glu	Trt G3 Glu m	Old	Young	1.95	1.78	33.00	1.10	0.28
T8	5	AgeCat*Trt*Glu	Trt G3 Glu p	Old	Young	-0.10	1.78	33.00	-0.06	0.95
T8	5	AgeCat*Trt*Glu	Trt G4 Glu m	Old	Young	-0.77	1.78	33.00	-0.43	0.67
T8	5	AgeCat*Trt*Glu	Trt G4 Glu p	Old	Young	1.15	1.78	33.00	0.65	0.52
T8	5	AgeCat*Trt*Glu	Trt G5 Glu m	Old	Young	1.19	1.78	33.00	0.67	0.51
T8	5	AgeCat*Trt*Glu	Trt G5 Glu p	Old	Young	0.68	1.78	33.00	0.39	0.70
T9	5	AgeCat*Trt*Glu	Trt G0 Glu m	Old	Young	-0.75	1.99	25.36	-0.38	0.71
T9	5	AgeCat*Trt*Glu	Trt G0 Glu p	Old	Young	-0.35	1.99	25.36	-0.18	0.86
T9	5	AgeCat*Trt*Glu	Trt G1 Glu m	Old	Young	-1.02	1.99	25.36	-0.51	0.61
T9	5	AgeCat*Trt*Glu	Trt G1 Glu p	Old	Young	-2.24	1.99	25.36	-1.12	0.27
T9	5	AgeCat*Trt*Glu	Trt G2 Glu m	Old	Young	-0.75	1.99	25.36	-0.38	0.71
T9	5	AgeCat*Trt*Glu	Trt G2 Glu p	Old	Young	-3.92	1.99	25.36	-1.97	0.06
T9	5	AgeCat*Trt*Glu	Trt G3 Glu m	Old	Young	-0.77	1.99	25.36	-0.39	0.70
T9	5	AgeCat*Trt*Glu	Trt G3 Glu p	Old	Young	-2.30	1.99	25.36	-1.15	0.26
T9	5	AgeCat*Trt*Glu	Trt G4 Glu m	Old	Young	-0.58	1.99	25.36	-0.29	0.77
T9	5	AgeCat*Trt*Glu	Trt G4 Glu p	Old	Young	-2.02	1.99	25.36	-1.01	0.32
T9	5	AgeCat*Trt*Glu	Trt G5 Glu m	Old	Young	0.08	1.99	25.36	0.04	0.97
T9	5	AgeCat*Trt*Glu	Trt G5 Glu p	Old	Young	-1.27	1.99	25.36	-0.64	0.53

APPENDIX II: cAMP DATA

cAMP **Project # a (2 Age groups, 6 Treatments, n = 27 horses)**

Sum Status: Summary statistics (mean, SD, se) for each group and treatment.

Age	Treatment	n	mean	SD	SE	min	median	max
Old	EGF +	10	1.0182	0.1547	0.0489	0.6300	1.0450	1.2022
Old	EGF -	10	1.0776	0.1838	0.0581	0.7672	1.0773	1.4418
Old	IGF-1 -	7	1.0743	0.1998	0.0755	0.8857	1.0493	1.4968
Old	IGF-1 +	7	1.2366	0.2088	0.0789	0.9963	1.2195	1.5210
Old	Insulin +	18	1.0645	0.1735	0.0409	0.7997	1.0321	1.4669
Old	Insulin -	18	1.0507	0.0941	0.0222	0.9265	1.0211	1.2465
Young	EGF +	5	1.0678	0.0444	0.0199	1.0206	1.0603	1.1225
Young	EGF -	5	1.1478	0.1399	0.0626	1.0125	1.0968	1.3161
Young	IGF-1 -	3	1.0783	0.0869	0.0502	0.9799	1.1104	1.1446
Young	IGF-1 +	3	1.0961	0.1545	0.0892	0.9177	1.1828	1.1878
Young	Insulin +	9	1.0324	0.0501	0.0167	0.9514	1.0416	1.0971
Young	Insulin -	9	0.9814	0.0799	0.0266	0.8347	1.0085	1.0529

Test 1: For each treatment, groups are compared. The Slice, Age (or Group) and_ Age (or_ Group) columns indicate the comparison. The Estimate column shows the estimated difference between the mean ratios for each comparison.

Slice	Age	_Age	Estimate	StdErr	DF	tValue	Rawp
Treatment EGF +	Old	Young	-0.04962	0.078767	92	-0.62993	0.530303
Treatment EGF -	Old	Young	-0.07026	0.078767	92	-0.89195	0.374745
Treatment IGF-1 -	Old	Young	-0.00401	0.099237	92	-0.04043	0.967839
Treatment IGF-1 +	Old	Young	0.140509	0.099237	92	1.415884	0.160186
Treatment Insulin +	Old	Young	0.032102	0.05871	92	0.546795	0.585843
Treatment Insulin -	Old	Young	0.069343	0.05871	92	1.181122	0.240599

Test 2: For each group, treatments are compared using Tukey's method. The Slice, Treatment and _Treatment columns indicate the comparison. The Estimate column shows the estimated difference between the mean ratios for each comparison that are significant at the 0.05 level are highlighted in pink.

Slice	Treatment	Treatment	Estimate	SE	DF	t value	Raw p	Adjustment	Adj p
Age Old	EGF +	EGF -	-0.059	0.064	92	-0.923	0.358	Tukey-K	0.940
Age Old	EGF +	IGF-1 -	-0.056	0.071	92	-0.792	0.431	Tukey-K	0.968
Age Old	EGF +	IGF-1 +	-0.218	0.071	92	-3.082	0.003	Tukey-K	0.031
Age Old	EGF +	Insulin +	-0.046	0.057	92	-0.816	0.416	Tukey-K	0.964
Age Old	EGF +	Insulin -	-0.033	0.057	92	-0.574	0.568	Tukey-K	0.993
Age Old	EGF -	IGF-1 -	0.003	0.071	92	0.046	0.963	Tukey-K	1.000
Age Old	EGF -	IGF-1 +	-0.159	0.071	92	-2.244	0.027	Tukey-K	0.228
Age Old	EGF -	Insulin +	0.013	0.057	92	0.230	0.818	Tukey-K	1.000
Age Old	EGF -	Insulin -	0.027	0.057	92	0.473	0.637	Tukey-K	0.997
Age Old	IGF-1 -	IGF-1 +	-0.162	0.077	92	-2.112	0.037	Tukey-K	0.290
Age Old	IGF-1 -	Insulin +	0.010	0.064	92	0.153	0.879	Tukey-K	1.000
Age Old	IGF-1 -	Insulin -	0.024	0.064	92	0.368	0.714	Tukey-K	0.999
Age Old	IGF-1 +	Insulin +	0.172	0.064	92	2.687	0.009	Tukey-K	0.088
Age Old	IGF-1 +	Insulin -	0.186	0.064	92	2.902	0.005	Tukey-K	0.051
Age Old	Insulin +	Insulin -	0.014	0.048	92	0.287	0.774	Tukey-K	1.000
Age Young	EGF +	EGF -	-0.080	0.091	92	-0.880	0.381	Tukey-K	0.950
Age Young	EGF +	IGF-1 -	-0.010	0.105	92	-0.100	0.921	Tukey-K	1.000
Age Young	EGF +	IGF-1 +	-0.028	0.105	92	-0.270	0.788	Tukey-K	1.000
Age Young	EGF +	Insulin +	0.035	0.080	92	0.441	0.660	Tukey-K	0.998
Age Young	EGF +	Insulin -	0.086	0.080	92	1.078	0.284	Tukey-K	0.889
Age Young	EGF -	IGF-1 -	0.070	0.105	92	0.662	0.510	Tukey-K	0.986
Age Young	EGF -	IGF-1 +	0.052	0.105	92	0.492	0.624	Tukey-K	0.996
Age Young	EGF -	Insulin +	0.115	0.080	92	1.439	0.154	Tukey-K	0.703
Age Young	EGF -	Insulin -	0.166	0.080	92	2.075	0.041	Tukey-K	0.309
Age Young	IGF-1 -	IGF-1 +	-0.018	0.117	92	-0.152	0.880	Tukey-K	1.000
Age Young	IGF-1 -	Insulin +	0.046	0.096	92	0.479	0.633	Tukey-K	0.997
Age Young	IGF-1 -	Insulin -	0.097	0.096	92	1.011	0.315	Tukey-K	0.913
Age Young	IGF-1 +	Insulin +	0.064	0.096	92	0.665	0.508	Tukey-K	0.985
Age Young	IGF-1 +	Insulin -	0.115	0.096	92	1.197	0.234	Tukey-K	0.837
Age Young	Insulin +	Insulin -	0.051	0.068	92	0.753	0.454	Tukey-K	0.975

cAMP **Project # b (4 Age-Diet groups, 6 Treatments, n = 19 horses)**

Sum Status: Summary statistics (Mean, SD, SE) for each group and treatment.

Group	Treatment	n	mean	SD	SE	min	median	max
Old (-)	EGF +	4	1.0453	0.0785	0.0393	1.0009	1.0087	1.1629
Old (-)	EGF -	4	1.0932	0.0217	0.0109	1.0736	1.0897	1.1198
Old (-)	IGF-1 -	3	1.0096	0.0627	0.0362	0.9470	1.0095	1.0725
Old (-)	IGF-1 +	3	1.0216	0.0282	0.0163	1.0037	1.0069	1.0541
Old (-)	Insulin +	7	0.9740	0.0917	0.0347	0.7799	0.9926	1.0643
Old (-)	Insulin -	7	1.0126	0.0109	0.0041	0.9964	1.0092	1.0273
Old (+)	EGF +	6	1.1324	0.1001	0.0409	1.0339	1.1087	1.3014
Old (+)	EGF -	6	1.1793	0.1711	0.0699	1.0059	1.1272	1.4377
Old (+)	IGF-1 -	4	1.1327	0.2161	0.1080	1.0088	1.0329	1.4562
Old (+)	IGF-1 +	4	1.2805	0.2178	0.1089	1.0652	1.2565	1.5438
Old (+)	Insulin +	8	1.3125	0.2532	0.0895	1.0304	1.3082	1.7840
Old (+)	Insulin -	8	1.0969	0.0961	0.0340	1.0161	1.0643	1.2452
Young (-)	EGF +	2	1.0324	0.0784	0.0554	0.9769	1.0324	1.0878
Young (-)	EGF -	2	1.0033	0.0324	0.0229	0.9804	1.0033	1.0262
Young (-)	IGF-1 -	1	0.9946	-	-	0.9946	0.9946	0.9946
Young (-)	IGF-1 +	1	1.0543	-	-	1.0543	1.0543	1.0543
Young (-)	Insulin +	2	0.9988	0.0812	0.0574	0.9413	0.9988	1.0562
Young (-)	Insulin -	2	1.0297	0.1416	0.1001	0.9296	1.0297	1.1298
Young (+)	EGF +	2	1.2624	0.1215	0.0859	1.1764	1.2624	1.3483
Young (+)	EGF -	2	1.1200	0.0292	0.0206	1.0994	1.1200	1.1407
Young (+)	Insulin +	2	1.0975	0.0401	0.0284	1.0691	1.0975	1.1259
Young (+)	Insulin -	2	1.0270	0.0152	0.0107	1.0162	1.0270	1.0377

Test 1: For each treatment, groups are compared. The Slice, Age (or Group) and_ Age (or_ Group) columns indicate the comparison. The Estimate column shows the estimated difference between the mean ratios for each comparison. (Diets) both raw (Raw p) and Tukey adjusted (Adj p) p- values are provided. Comparisons that are significant at the 0.05 level are highlighted in pink.

Slice	Group	Group	Esti	SE	DF	t value	Rawp	Adjust	Adj p
Treatment EGF +	Old (-)	Old (+)	-0.09	0.08	39.31	-1.09	0.284	Tukey-K	0.704
Treatment EGF +	Old (-)	Young (-)	0.01	0.07	5.87	0.18	0.863	Tukey-K	0.998
Treatment EGF +	Old (-)	Young (+)	-0.22	0.06	7.81	-3.90	0.005	Tukey-K	0.010
Treatment EGF +	Old (+)	Young (-)	0.10	0.10	17.31	1.01	0.325	Tukey-K	0.745
Treatment EGF +	Old (+)	Young (+)	-0.13	0.09	26.88	-1.48	0.150	Tukey-K	0.477
Treatment EGF +	Young (-)	Young (+)	-0.23	0.08	7.26	-2.87	0.023	Tukey-K	0.059
Treatment EGF -	Old (-)	Old (+)	-0.09	0.08	39.31	-1.07	0.289	Tukey-K	0.711
Treatment EGF -	Old (-)	Young (-)	0.09	0.07	5.87	1.25	0.258	Tukey-K	0.607
Treatment EGF -	Old (-)	Young (+)	-0.03	0.06	7.81	-0.48	0.643	Tukey-K	0.962
Treatment EGF -	Old (+)	Young (-)	0.18	0.10	17.31	1.78	0.092	Tukey-K	0.328
Treatment EGF -	Old (+)	Young (+)	0.06	0.09	26.88	0.68	0.505	Tukey-K	0.904
Treatment EGF -	Young (-)	Young (+)	-0.12	0.08	7.26	-1.46	0.187	Tukey-K	0.491
Treatment IGF-1(-)	Old (-)	Old (+)	-0.12	0.10	38.37	-1.26	0.214	Tukey-K	0.441
Treatment IGF-1 (-)	Old (-)	Young (-)	0.02	0.10	5.22	0.15	0.884	Tukey-K	0.987
Treatment IGF-1 (-)	Old (+)	Young (-)	0.14	0.13	13.88	1.07	0.304	Tukey-K	0.551
Treatment IGF-1 +	Old (-)	Old (+)	-0.26	0.10	38.37	-2.66	0.011	Tukey-K	0.051
Treatment IGF-1 +	Old (-)	Young (-)	-0.03	0.10	5.22	-0.33	0.753	Tukey-K	0.941
Treatment IGF-1 +	Old (+)	Young (-)	0.23	0.13	13.88	1.75	0.102	Tukey-K	0.228
Treatment Insulin +	Old (-)	Old (+)	-0.34	0.07	37.26	-4.96	0.000	Tukey-K	0.002
Treatment Insulin +	Old (-)	Young (-)	-0.02	0.07	5.04	-0.36	0.734	Tukey-K	0.983
Treatment Insulin +	Old (-)	Young (+)	-0.12	0.05	6.09	-2.37	0.055	Tukey-K	0.136
Treatment Insulin +	Old (-)	Young (-)	0.31	0.09	13.88	3.43	0.004	Tukey-K	0.023
Treatment Insulin +	Old (+)	Young (+)	0.21	0.08	22.67	2.70	0.013	Tukey-K	0.079
Treatment Insulin +	Young (-)	Young (+)	-0.10	0.08	7.26	-1.23	0.256	Tukey-K	0.619
Treatment Insulin -	Old (-)	Old (+)	-0.08	0.07	37.26	-1.24	0.224	Tukey-K	0.618
Treatment Insulin -	Old (-)	Young (-)	-0.02	0.07	5.04	-0.25	0.814	Tukey-K	0.994
Treatment Insulin -	Old (-)	Young (+)	-0.01	0.05	6.09	-0.28	0.791	Tukey-K	0.992
Treatment Insulin -	Old (+)	Young (-)	0.07	0.09	13.88	0.74	0.475	Tukey-K	0.881
Treatment Insulin -	Old (+)	Young (+)	0.07	0.08	22.67	0.88	0.388	Tukey-K	0.815
Treatment Insulin -	Young (-)	Young (+)	0.00	0.08	7.26	0.03	0.974	Tukey-K	1.000

Test 2: For each group, treatments are compared using Tukey's method. The Slice, Treatment and _Treatment columns indicate the comparison. The Estimate column shows the estimated difference between the mean ratios for each comparison that are significant at the 0.05 level are highlighted in pink.

Slice	Treat	Treat	Estim	SE	DF	t value	Raw p	Adj	Adj p
Old (-)	EGF +	EGF -	-0.048	0.043	22	-1.119	0.275	Tukey-K	0.86434
Old (-)	EGF +	IGF-1 -	0.036	0.046	22	0.772	0.449	Tukey-K	0.96731
Old (-)	EGF +	IGF-1 +	0.024	0.046	22	0.514	0.613	Tukey-K	0.99457
Old (-)	EGF +	Insulin +	0.071	0.038	22	1.879	0.074	Tukey-K	0.45777
Old (-)	EGF +	Insulin -	0.033	0.038	22	0.863	0.397	Tukey-K	0.94837
Old (-)	EGF -	IGF-1 -	0.084	0.046	22	1.808	0.084	Tukey-K	0.4961
Old (-)	EGF -	IGF-1 +	0.072	0.046	22	1.550	0.135	Tukey-K	0.64241
Old (-)	EGF -	Insulin +	0.119	0.038	22	3.142	0.005	Tukey-K	0.07233
Old (-)	EGF -	Insulin +	0.081	0.038	22	2.126	0.045	Tukey-K	0.33712
Old (-)	IGF-1 -	IGF-1 +	-0.012	0.049	22	-0.241	0.811	Tukey-K	0.99985
Old (-)	IGF-1 -	Insulin +	0.036	0.042	22	0.853	0.403	Tukey-K	0.95087
Old (-)	IGF-1 -	Insulin -	-0.003	0.042	22	-0.070	0.945	Tukey-K	1
Old (-)	IGF-1 +	Insulin +	0.048	0.042	22	1.138	0.267	Tukey-K	0.85634
Old (-)	IGF-1 +	Insulin -	0.009	0.042	22	0.216	0.831	Tukey-K	0.99992
Old (-)	Insulin +	Insulin -	-0.039	0.032	22	-1.191	0.246	Tukey-K	0.83294
Old (+)	EGF +	EGF -	-0.047	0.105	30	-0.447	0.658	Tukey-K	0.99715
Old (+)	EGF +	IGF-1 -	0.000	0.117	30	-0.003	0.998	Tukey-K	1
Old (+)	EGF +	IGF-1 +	-0.148	0.117	30	-1.262	0.217	Tukey-K	0.79912
Old (+)	EGF +	Insulin +	-0.180	0.098	30	-1.833	0.077	Tukey-K	0.48213
Old (+)	EGF +	Insulin -	0.035	0.098	30	0.361	0.720	Tukey-K	0.99896
Old (+)	EGF -	IGF-1 -	0.047	0.117	30	0.397	0.694	Tukey-K	0.99837
Old (+)	EGF -	IGF-1 +	-0.101	0.117	30	-0.862	0.396	Tukey-K	0.9487
Old (+)	EGF -	Insulin +	-0.133	0.098	30	-1.355	0.185	Tukey-K	0.75086
Old (+)	EGF -	Insulin -	0.082	0.098	30	0.839	0.408	Tukey-K	0.95387
Old (+)	IGF-1 -	IGF-1 -	-0.148	0.129	30	-1.149	0.259	Tukey-K	0.85153
Old (+)	IGF-1 -	Insulin +	-0.180	0.111	30	-1.614	0.117	Tukey-K	0.60549
Old (+)	IGF-1 -	Insulin -	0.036	0.111	30	0.322	0.750	Tukey-K	0.99941
Old (+)	IGF-1 +	Insulin +	-0.032	0.111	30	-0.287	0.776	Tukey-K	0.99966
Old (+)	IGF-1 +	Insulin -	0.184	0.111	30	1.649	0.110	Tukey-K	0.58564
Old (+)	Insulin +	Insulin -	0.216	0.091	30	2.371	0.024	Tukey-K	0.24063
Young (-)	EGF +	EGF -	0.029	0.092	4	0.316	0.768	Tukey-K	0.99946
Young (-)	EGF +	IGF-1 -	0.038	0.113	4	0.335	0.754	Tukey-K	0.99928
Young (-)	EGF +	IGF-1 +	-0.022	0.113	4	-0.194	0.855	Tukey-K	0.99995
Young (-)	EGF +	Insulin +	0.034	0.092	4	0.366	0.733	Tukey-K	0.9989
Young (-)	EGF +	Insulin -	0.003	0.092	4	0.029	0.978	Tukey-K	1

Young (-)	EGF -	IGF-1 +	0.009	0.113	4	0.078	0.942	Tukey-K	1
Young (-)	EGF -	IGF-1 +	-0.051	0.113	4	-0.452	0.675	Tukey-K	0.997
Young (-)	EGF -	Insulin +	0.005	0.092	4	0.050	0.963	Tukey-K	1
Young (-)	EGF -	Insulin +	-0.026	0.092	4	-0.287	0.789	Tukey-K	0.99966
Young (-)	IGF-1 -	EGF +	-0.060	0.130	4	-0.459	0.670	Tukey-K	0.99679
Young (-)	IGF-1 -	Insulin +	-0.004	0.113	4	-0.037	0.972	Tukey-K	1
Young (-)	IGF-1 -	Insulin -	-0.035	0.113	4	-0.312	0.771	Tukey-K	0.99949
Young (-)	IGF-1 +	Insulin +	0.056	0.113	4	0.493	0.648	Tukey-K	0.99551
Young (-)	IGF-1 +	Insulin -	0.025	0.113	4	0.218	0.838	Tukey-K	0.99991
Young (-)	Insulin +	Insulin -	-0.031	0.092	4	-0.336	0.754	Tukey-K	0.99927
Young (+)	EGF +	EGF -	0.142	0.066	4	2.154	0.097	Tukey-K	0.19187
Young (+)	EGF +	Insulin +	0.165	0.066	4	2.496	0.067	Tukey-K	0.11175
Young (+)	EGF +	Insulin +	0.235	0.066	4	3.564	0.024	Tukey-K	0.01793
Young (+)	EGF -	Insulin +	0.023	0.066	4	0.341	0.750	Tukey-K	0.98566
Young (+)	EGF -	Insulin -	0.093	0.066	4	1.409	0.232	Tukey-K	0.5177
Young (+)	Insulin +	Insulin -	0.071	0.066	4	1.068	0.346	Tukey-K	0.71451

LIST OF ABBREVIATIONS

AMPK	Adenosine monophosphate-activated protein
PBS	Phosphate buffer saline
LH	Luteinizing hormone
LHR	luteinizing hormone receptor
FSH	Follicle stimulating hormone
FSHR	Follicle-stimulating hormone receptor
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
IGF-1	Insulin like growth factor
IGF-1R	Insulin like growth factor receptor
hCG	Human chorionic gonadotropin
GLUT4	Glucose transporter 4
PCOS	Poly cystic ovarian fibrosis
cAMP	Cyclic Activated protein kinase
GEF	Epac1 Guanine-nucleotide-exchange factor Epac1
DEP	Dishevelled, Egl-10, Pleckstrin
GDF-9	Growth differentiation factor-9
BMP-15	Bone morphogenetic protein-15
PDGF	Platelet derived growth factor
AMP	Activated protein kinase
ATP	Adenosine triphosphate
MS	Metabolic syndrome
EMS	Equine metabolic syndrome
PPID	Pituitary pars intermediate dysfunction

TSC2	Tuberous sclerosis complex2
ERK	Extracellular signal-regulated kinase
IR	Insulin receptor
p62TCF	phosphorylation of transcription factors
mRNA	Messenger RNA
GDP	Guanosine diphosphate
GTP	Guanosine triphosphate
CFP	Cyan fluorescent protein
YFP	Yellow fluorescent protein
GPCR	G protein coupled receptor
Gs	Stimulatory G protein
RT-PCR	Real-Time real- time reverse transcriptase polymerase chain reaction
PCR	Polymerase chain reaction
cDNA	Complementary DNA
DNA	Deoxy Nucleic Acid
RNA	Ribose Nucleic Acid
PKA	Protein Kinase A
GEF	Guanine nucleotide exchange factors
Di-4-ANPPDHQ	Aminonaphthylethenylpyridinium
P	Progesterone
IU	International Units
IV	Intravenous
DMEM	Dulbecco's Modified minimum essential medium
EDTA	Ethylene diamine tetra acetic acid
RANase	Ribonuclease
DNAase	Deoxyribonuclese
HKG	Housekeeping gene

GAPDH	Glyceraldehyde-3 phosphate dehydrogenase
ACTB	Beta-actin
SDHA	Succinate dehydrogenase complex
Ct	Cycle threshold
ΔCt	Delta cycle threshold
Δ ΔCt	Double Delta Ct
ICUE3	DNA plasmid
OPTI-MEM	Reduced serum medium
PAS	Phosphate Buffered Solutions
BSA	Bovine serum albumin
FRET	Fluorescence energy transfer