ODL, ours shows high pup survival when the mothers are maintained on a chow diet and no change in % biliary hyodeoxycholate. Further, both male and female kos are hypercholesterolemic compared with wild type: 166±14 vs 107±9 mg choles/dl, p<.003, and 140±10 vs 95±12 mg choles/dl, p<.01, respectively. By gradient gel electrophoresis of plasma followed by oil red O staining, the major difference in the lipoprotein patterns was in the distribution of LDL-size particles(ps): In the kos, the smallest ps (IVA+IVB) were increased 56% in males, p<.002, and 83% in females, p<.001, while the largest size ps(I and IIA+IIB) were reduced 33% in both, p<.001. All kos were normotriglyceridemic, normoglycemic and showed no change in plasma free fatty acids relative to wild-type; however, male kos were hyperinsulinemic, 3.28±0.53 vs 0.95±0.30 ug insulin/l, p<.04. Absence of hyperinsulinemia in female kos suggests that the altered LDL pattern observed in both genders is not solely a function of plasma insulin level. Total fecal bile acids (BA) were decreased 75% in male kos and 56% in female kos. Gall bladder BA distribution in male kos showed 41% decrease in cholate, p<.002 and 2.2 fold increase in β-muricholate, p<.002,but female kos showed little change from wild type. The data taken together support a pivotal role for cyp7A in plasma cholesterol and lipoprotein homeostasis and suggest the possibility that cyp7A and/or bile acids affect insulin homeostasis in a gender-specific fashion.

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### Cafestol, the Cholesterol-Raising Factor in Boiled Coffee, Increases Serum Cholesterol Levels in Apolipoprotein E*3-Leiden Transgenic Mice by Suppression of Bile Acid Synthesis

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Cafestol, a diterpene present in boiled coffee, potently increases serum cholesterol (Chol) levels in humans. So far, no suitable animal model has been found to study the biochemical background of this effect. We determined the effect of cafestol on serum Chol in different mice strains. Serum Chol levels were significantly increased in ApoE*3-Leiden, LDLR+/- and WT mice fed a cafestol (0.05% w/w) diet for 8 weeks (61%,55% and 46%, respectively), mainly due to a rise in VLDL- and IDL-Chol in all three strains. The mechanism of action of the Chol-raising effect was further studied in apoE*3-Leiden mice fed a cafestol (0.05% w/w) or placebo diet for 3 weeks. Cafestol suppressed enzyme activity (-57%) and mRNA levels (-58%) of Chol 7α-hydroxylase. mRNA levels of enzymes involved in the alternative pathway of bile acid synthesis (BAS) i.e. sterol 27-hydroxylase and oxysterol 7α-hydroxylase were reduced, by 32% and 48%, respectively. The total amount of bile acids secreted in feces was decreased by 41%. Cafestol did not affect hepatic free and esterified Chol, but it decreased LDLR mRNA levels by 37%. VLDL particles contained a 3-fold higher amount of Chol-esters, indicative for the secretion of a βVLDL-like particle. In line with this, VLDL-triglyceride (TG) production was decreased in cafestol treated mice as compared to placebo as a result of a reduction in hepatic TG content by 52%. In conclusion, cafestol increases serum Chol levels in apoE*3 Leiden transgenic mice by suppression of the major regulatory enzymes in the BAS pathways, leading to decreased LDLR mRNA levels and increased secretion of Chol-esters by the liver. We suggest that suppression of (BAS) may provide an explanation for the Chol-raising effect of cafestol in humans.

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### Altered Hepatic Cholesterol Metabolism in Peroxisome-Proliferator-Activated Receptor *Alpha*-Deficient Mice

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The transcription factor peroxisome proliferator-activated receptor *alpha* (PPARα) controls hepatic expression of genes involved in regulation of lipoprotein metabolism (apo A-I, C-III) and fatty acid oxidation in response to endogenous (fatty acids) and exogenous (fibrates) activators. PPARα-deficiency results in hypercholesterolemia and hepatic triglyceride accumulation in chow-fed mice. It is becoming increasingly clear that hepatic fatty acid and cholesterol metabolism are coordinately regulated through the actions of another family of transcription factors, designated sterol regulatory element-binding proteins (SREBP). To evaluate the interactions between both systems, we have studied the expression of key genes in the regulation of hepatic cholesterol metabolism in untreated and ciprofibrate-treated PPARα-deficient (-/-) and control (+/+) mice. The hepatic free cholesterol content is not affected while the amount of cholesterylester is 4-times higher in (-/-) mice compared to (+/+) controls. Ciprofibrate does not change hepatic cholesterol content in (-/-) mice but causes a doubling of cholesterylesters in (+/+) livers. RT-PCR analyses revealed that the expression of *SREBP1a* (-85%, normalized to β-actin) and *SREBP2* (-50%) is strongly reduced in untreated (-/-) mice when compared to (+/+) mice, with a concommitant reduction in HMGCoA reductase (-75%) and LDL receptor (-70%) mRNA levels. In addition, expression of *mdr2*, encoding mdr2 P-glycoprotein that is essential for biliary cholesterol secretion, is reduced by 53% in (-/-) mice and is not induced by ciprofibrate, as is the case in (+/+) mice. These data indicate that PPAR -defici[illegible] ff[illegible] [illegible]ratic uptake [illegible]rth sis and biliary secretion of cholesterol in mice,

# Arteriosclerosis, Thrombosis & Vascular Biology, Basic Cardiovascular Sciences:

## Cardiac Developmental Biology II

*Wednesday Morning*

*Georgia World Congress Center Exhibit Hall*

*Abstracts 3623–3631*

3623

### A Striated-Muscle Isoform of β1 Integrin Is Involved in Cardiac Hypertrophy and Activation of Focal Adhesion Kinase but Not Cardiac Cell-Cycle Arrest

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Integrins are heterodimeric cell-surface receptors that can mechanotransduce extracellular stimuli and trigger intracellular biochemical signals. Because their roles in matrix-cell interaction during cardiac growth are poorly understood, this study investigated a striated-muscle isoform of β1 integrin, termed β1D, and its roles in cardiac hypertrophy, activation of focal adhesion kinase (FAK), organization of the myocyte cytoskeleton / myofibril, and its ability to modulate cardiac cell-cycling. α-adrenergic stimulation of neonatal rat ventricular myocytes (NRVM) with $10^{-4}$M phenylephrine (PE) increased activities of both atrial natriuretic factor (ANF) and myosin light chain-2 ventricular promoter-luciferase reporters in a matrix dependent manner. PE induced rapid and sustained phosphorylation of FAK in NRVM and also significantly increased expression of β1D protein. β1D-specific immunostaining revealed that PE redirects β1D integrin to Z-lines in NRVM. Infection of NRVM was performed with recombinant adenoviruses which overexpress β1D or control (lacZ) transgenes driven by the strong cytomegalovirus promoter. β1D overexpression resulted in: 1)increased ANF promoter-luciferase activity in serum-free conditions by 2-3 fold, 2)increased myofibrillar organization, and 3)increased phosphorylation of FAK. However, β1D did not change the phosphorylation state of ERK1/ERK2. Contrary to previously published work in skeletal muscle, β1D did not promote withdrawal from cell-cycling, as assessed by BrdU incorporation and immunostaining experiments in both neonatal and embryonic (day 15-16 post-coitum) rat ventricular myocyte cultures. **Conclusion:** β1D integrin is involved in hypertrophic growth and FAK activation but not cell-cycle arrest of cardiac myocytes.

3624

### Negative Control of Hypertrophy-Sensitive Gene Expression by a Tetracycline-Inducible $Ca^{2+}$/Calmodulin-Dependent Protein Kinase II Expression System

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Recently, it has been shown that intracellular calcium controls molecular mechanisms that activate cardiac hypertrophy. This process appears to be modulated through the activities of three key $Ca^{2+}$/CaM-dependent enzymes: calcineurin (CaN), CaM KIV, and CaM KII. CaN and/or CaM KIV transcriptionally up-regulate many hypertrophy-sensitive genes including: atrial natriuretic factor (ANF), cardiac α-actin, and skeletal α-actin. This laboratory has demonstrated that active CaM KII, in the cardiomyocyte, negatively regulates the entire genetic program for hypertrophy-sensitive gene induction. Moreover, CaM KII completely silenced hypertrophic gene expression when co-expressed with virally-driven active CaN and/or CaM KIV. In order to regulate cardiomyocyte expression of exogenous CaM KII, we subcloned CaM KII downstream of a minimal promoter under the transcriptional control of the tetracycline-regulated transactivator (tTA). In the presence of active CaN and/or CaM KIV, tTA-regulated CaM KII completely silenced (> 40% below baseline) each of three hypertrophy-sensitive genes. Doxycycline (DOX) exposure to cultured cardiomyocytes blocked tTA mediated CaM KII silencing as expected and restored CaN and CaM KIV mediated induction of reporter activities. Taken together, results from this study demonstrate that the regulated expression of CaM KII alpha isoforms negatively controls hypertrophy-sensitive gene expression in the cardiomyocyte. Negative control can be maintained and operated in a dominant fashion even under conditions when transcriptional induction cascades are being driven by constitutively-active expressed components.

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### Transcriptional Regulation of the Chick Nkx-2.5 Gene in Early Cardiac Development

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Specification of early precardiac mesoderm fate in the appropriate regions of the chick embryo occurs due to inductive effect of signals from the anterior endoderm on gastrulating mesoderm. These signals include BMPs or bone morphogenetic proteins, members of the diverse TGF-β class of secreted signalling molecules; and antagonists of the Wnt signalling pathway. These secreted signals specify mesodermal induction of the cardiac gene program in lateral and anterior regions of the embryo, respectively. One of the earliest cardiac genes activated by these signals is Nkx-2.5, a homeodomain transcription factor whose homolog has been found to be the earliest expressed marker of specified precardiac mesoderm in every vertebrate species studied thus far. Vertebrate Nkx-2.5 genes are homologs of the Drosophila tinman gene, which was found to be absolutely required for the development of the dorsal aorta, the contractile vessel carrying out cardiac function in flies. Deletion and overexpression experiments carried out with Nkx-2.5 suggest that it may, along with other Nkx tinman homologs, to