

MEETING ABSTRACTS

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Metabolism, diet and disease

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ORAL PRESENTATIONS

O1

Obesity, insulin resistance, and the pathway to type 2 diabetes

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The increase in obesity in the U.S. and elsewhere can be explained by a variety of factors, as outlined by David Allison and his colleagues. Not only is the fast food diet a factor, but also are changes in ambient temperature, obesity-causing psychoactive molecules, associative sorting, changes in smoking habits, and reduction in sleep. In particular is the increase in visceral fat, which is associated with insulin resistance. We have shown in experimental animals that omentectomy increases insulin sensitivity implicating visceral fat *per se* as an important factor. We have followed longitudinally changes in organ function and crosstalk in a large animal model, rendered overweight by a high fat, hypercaloric diet. In normal animals, like in normal humans, there is a wide range of adiposity as well as visceral and subcutaneous fat deposition. Feeding fat increases visceral and subcutaneous fat with little change in body weight. Physiologic changes occur with absolutely no change in fasting glucose: most predictive of insulin resistance is relative reduction in metabolic clearance of insulin by liver, and a modest increase in beta cell sensitivity to glucose. The latter changes result in hyperinsulinemia. Change in insulin sensitivity is also predicted by change in liver insulin clearance, implicating metabolic clearance of insulin as the primary factor in development of the insulin resistance syndrome.

O2

PI 3-kinase and disease

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Phosphoinositide 3-Kinase (PI3K) is a central enzyme in a signaling pathway that mediates cellular responses to growth factors. This enzyme phosphorylates the 3 position of phosphatidylinositol-4,5-bisphosphate to produce phosphatidylinositol-3,4,5-trisphosphate (PIP₃) at the plasma membrane. A number of signaling proteins, including the Ser/Thr protein kinases, AKT and PDK1, contain pleckstrin homology domains that bind specifically to PIP₃. Thus, the generation of PIP₃ at the plasma membrane in response to activation of PI3K by growth factors results in the initiation of downstream Ser/Thr phosphorylation cascades that control a variety of cellular responses. The signaling pathway downstream of PI3K is highly conserved from worms and flies to humans and genetic analysis of the pathway has revealed a conserved role in regulating glucose metabolism and cell growth. Based on deletion of genes encoding the catalytic or regulatory subunits of PI3K in the mouse, PI3K mediates insulin dependent regulation of glucose metabolism, and defects in activation of this

pathway result in insulin resistance. In contrast, mutational events that lead to hyperactivation of the PI3K pathway result in cancers. Activating mutations in PIK3CA, encoding the p110α catalytic subunit of PI3K or inactivating mutations in PTEN, a phosphoinositide 3-phosphatase that reverses the effects of PI3K, are among the most common events in solid tumors. We have generated mouse models in which a mutated form of the PIK3CA gene is expressed in a tissue specific and reversibly inducible manner. These mice develop cancers that are dependent on continuous expression of the mutant PIK3CA gene. The PIK3CA driven tumors are FDG-PET positive and turning off PI 3-Kinase with PI3K inhibitors that are in human clinical trials results in an acute decline in FDG-PET signal that precedes tumor shrinkage. These results suggest that the ability of PI3K to stimulate high rates of glucose uptake and metabolism may be critical for the survival of PIK3CA mutant tumors. The role of PI3K inhibitors for treating cancers in mouse models and in human trials will be discussed.

O3

Effects of serotonin on skeletal muscle growth

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Background: Myostatin (MSTN), a member of the transforming growth factor beta family, negatively regulates skeletal muscle mass. Deletion of the *Mstn* gene results in increased muscle mass. Also, *Mstn*^{-/-} mice and mice expressing a dominant negative MSTN receptor (activin receptor type IIB, ACVR2B) show decreased adipose tissue. *Mstn*^{-/-} mice fed a high fat diet gain less weight, have improved glucose tolerance and are more sensitive to insulin when compared to wild type controls. We are interested in characterizing molecules that may be downstream of MSTN signaling. We identified serotonin as a potential molecule that may interact with MSTN in skeletal muscle. Serotonin is thought to regulate both metabolism and cardiac hypertrophy, but it is not clear if serotonin is synthesized and functions in the skeletal muscle. We hypothesize that serotonin regulates skeletal muscle mass and metabolism, and the serotonin and myostatin pathways interact with each other.

Materials and methods: Microarray screening was carried out on gastrocnemius muscle from transgenic mice carrying dominant negative ACVR2B and wild type littermates. Selected results were validated by real time qPCR (RT-qPCR) using cDNA from transgenic muscle and muscle from mice injected with a MSTN inhibitor (a soluble ACVR2B). Rat L6 myoblasts were differentiated in horse serum with or without serotonin. Myotube size was determined from anti-myosin heavy chain immunofluorescence using NIC Elements software (Nikon). Serotonin concentrations in transgenic muscle and L6 cells were determined by HPLC.

Results: Expression of tryptophan hydroxylase 1 (Tph1), the enzyme that catalyzes the rate limiting step in serotonin synthesis was significantly increased by microarray in transgenic mice and by RT-qPCR in mice receiving the soluble receptor. Serotonin significantly promoted longitudinal growth of skeletal muscle fibers *in vitro*. Increased branching, differentiation and fusion of myoblasts into myotubes were also observed. Serotonin was detectable in muscle from transgenic mice and in L6 myoblasts and myotubes.

Conclusions: This study is the first to show that serotonin is found in skeletal muscle, and promotes muscle growth *in vitro*. We also show that the *Tph1* enzyme is expressed in skeletal muscle, and that *Tph1* expression increases with hypertrophy after MSTN inhibition. To elucidate the mechanism by which MSTN and serotonin pathways interact with each other, and their role in muscle growth and insulin signaling, we are using specific agonists and antagonists along with RT-qPCR to determine which of the 15 serotonin receptors are expressed by skeletal muscle fibers in presence or absence of MSTN. Since *Mstn*^{-/-} mice show improved glucose tolerance, and serotonin has previously been implicated in insulin secretion and glucose uptake, we are interested in understanding how MSTN and serotonin may regulate glucose metabolism in skeletal muscle. We hope that this study will not only shed light on normal muscle development and metabolism, but will also suggest potential targets that could be manipulated therapeutically with respect to muscle wasting diseases, diabetes, and obesity.

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O4

Abstract not submitted for online publication

O5

Reconsidering the policy options to combat obesity

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As the prevalence of obesity steadily grows, so too does our understanding of the threats that excess weight poses to both our individual health and our nation as a whole. With this information, there is a need now more than ever to reconsider the options we have to mitigate the health risks and costs of this growing epidemic. This includes, among other conversations, examining how employers can play a role in encouraging healthier habits through workforce-based initiatives and a reconsideration of the risk-benefit paradigm that informs the decisions of payers, regulators, and providers on this crucial issue.

O6

Leptin and the homeostatic system regulating body weight

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The discovery of leptin has led to the elucidation of a robust physiologic system that maintains fat stores at a relatively constant level. Leptin is a peptide hormone secreted by adipose tissue in proportion to its mass. This hormone circulates in blood and acts on the hypothalamus to regulate food intake and energy expenditure. When fat mass falls, plasma leptin levels fall stimulating appetite and suppressing energy expenditure until fat mass is restored. When fat mass increases, leptin levels increase, suppressing appetite until weight is lost. By such a mechanism total energy stores are stably maintained within a relatively narrow range.

Recessive mutations in the leptin gene are associated with massive obesity in mice and some humans. Treatment with recombinant leptin markedly reduces food intake and body weight. The low leptin levels in patients with leptin mutations are also associated with multiple abnormalities including infertility, diabetes and immune abnormalities all of which are corrected by leptin treatment. These findings have established important links between energy stores and many other physiologic systems and led to the use of leptin as a treatment for an increasing number of other human conditions including a subset of obesity, some forms of diabetes including lipodystrophy and hypothalamic amenorrhea, the cessation of menstruation seen in extremely thin women. Identification of a physiologic system that controls energy balance establishes a biologic basis for obesity and further establishes links between leptin and numerous other physiologic responses. Recent studies have explored the relationship between leptin and the reward value of food. In addition, new methods for identifying neurons activated by leptin and other stimuli have been developed.

O7

Surviving starvation: essential role of the ghrelin-growth hormone axis

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Chronic starvation is a repeated threat to survival of animals of all species. Indeed, about 15% of the current human population is estimated to suffer from severe malnutrition, and one-half of all deaths in children less than 5 years of age (~6 million deaths per year) arise from malnutrition. Over the centuries, starvation has exerted profound evolutionary pressure that has selected for a variety of adaptive mechanisms to support life. Paramount among these mechanisms is the necessity to maintain blood sugar concentrations sufficient for brain function. The adaptive mechanisms are particularly strained when chronic starvation has depleted the body of its other source of energy – namely, fatty acids stored as triglycerides.

Recently, our laboratory has begun to study the adaptation to chronic starvation, focusing on the essential roles of two peptide hormones, ghrelin and growth hormone. Ghrelin, a peptide hormone secreted by neuroendocrine cells in the stomach, was identified in 1999 by Kojima and Kangawa by its ability to stimulate release of growth hormone. In rodents and humans, plasma ghrelin rises before meals and declines after eating. Administration of excess ghrelin increases food intake, but knockout mice lacking ghrelin or its receptor have normal weight. Therefore, the true function of ghrelin has been enigmatic. Ghrelin is unique in that it requires a covalently attached 8-carbon fatty acid for activity, a modification conserved in all vertebrates. We identified ghrelin O-acyltransferase (GOAT), the enzyme that attaches octanoate to ghrelin. GOAT knockout mice cannot produce active ghrelin. Like ghrelin knockouts, GOAT knockouts have normal body weight. When these knockout mice are placed on a 60% calorie-restricted diet for 8 days, they are unable to maintain normal blood glucose and die. Restoration of ghrelin or growth hormone prevents death. Thus, ghrelin

maintains blood glucose under conditions of severe calorie restriction, a function essential for survival in chronically starved rodents. Recent data on the mechanism by which the ghrelin-growth hormone axis preserves blood glucose will be presented.

08

Sirtuins, aging and diseases

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SIRT2 and related genes (sirtuins) are NAD-dependent deacetylases that link metabolism, protein acetylation and aging in a variety of species. Sirtuins are also involved in the longevity conferred by dietary or calorie restriction (CR). The mammalian sirtuins SIRT1-7 are involved in changes in stress resistance and metabolism that are triggered by CR, which not only extends life span, but also protects against many diseases of aging. In this talk, I will describe how mammalian SIRT1 impacts tissue metabolism and diseases by deacetylating nuclear transcription factors that govern key physiological pathways. I will also describe new data from several labs showing that the mitochondrial sirtuin SIRT3 suppresses reactive oxygen species (ROS) in mitochondria and thus links sirtuins, calorie restriction, ROS, mitochondria and aging. The effect of SIRT3 on mitochondrial generated ROS further regulates the key nuclear transcription factor HIF-1 α , and may thus govern the metabolic reprogramming (Warburg effect) in cancer. Finally, I will touch upon recent progress in trying to understand the mechanism of action of SIRT1-activating compounds, which are currently in numerous human clinical trials.

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09

Regulation of metabolism by sirtuins

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Mitochondrial sirtuins are NAD-dependent enzymes that bind and regulate numerous metabolic pathways within the mitochondria. For example, SIRT3 functions as an NAD-dependent deacetylase that binds and activates numerous oxidative pathways. We have discovered that sirtuins regulate metabolic pathways important in tumor cell metabolism. One hallmark feature of tumor cells is a shift from oxidative to glycolytic metabolism, and this reliance on aerobic glycolysis to support cell growth is known as the Warburg effect. We have discovered that SIRT3 has an additional effect on cellular metabolism by repressing cellular glycolysis through the regulation of HIF1 α , a transcription factor that increases gene expression of glycolytic targets. SIRT3 null cells exhibit metabolic and genetic features of the Warburg effect and enhanced tumorigenicity *in vivo*. Likewise, SIRT3 overexpression reduces glycolysis in tumor cells. In sum, a better understanding of sirtuin-mediated regulation may identify novel ways to therapeutically target tumor metabolism.

010

AMPK - a nutrient and energy sensor with roles in diabetes, cancer and viral infection

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The AMP-activated protein kinase (AMPK) is a cellular energy sensor that is conserved throughout the eukaryotic domain. AMPK exists as complexes comprising catalytic α subunits and regulatory β and γ subunits, which are activated >100-fold by phosphorylation of the α subunit at Thr-172 by upstream kinases. Binding of AMP to one site on the γ subunit causes 10-fold allosteric activation by AMP, while binding of either AMP or ADP to a second site promotes phosphorylation

and inhibits dephosphorylation of Thr-172. Since both effects are antagonized by ATP, the kinase is activated by falling cellular energy status, when it phosphorylates downstream targets that switch on catabolic pathways generating ATP, while switching off processes consuming ATP. Catabolic processes switched on including uptake and metabolism of glucose and fatty acids, and mitochondrial biogenesis. ATP-consuming processes switched off include biosynthetic pathways for lipids (fatty acids, phospholipids, cholesterol), carbohydrates (gluconeogenesis, glycogen synthesis), proteins and ribosomal RNA, and progress through the cell cycle.

By switching on glucose and fatty acid uptake and metabolism and switching off gluconeogenesis, AMPK should reverse the key metabolic defects in insulin resistance and type 2 diabetes. Consistent with this, AMPK is a target, although probably not the only target, for the anti-diabetic drug metformin. Because AMPK activation inhibits most biosynthetic pathways and causes cell cycle arrest, it should also have a cytostatic, tumor suppressor effect. The discovery in 2003 that the major upstream kinase that phosphorylates Thr-172 is the tumor suppressor kinase, LKB1, strengthened this view. The finding also led to retrospective analyses suggesting that metformin protects against the development of cancer.

If AMPK is a tumor suppressor, one would expect that it would be down-regulated in many tumors. This indeed occurs through loss-of-function mutations in the upstream kinase LKB1, particularly in non-small cell lung cancer and cervical cancer. The majority of cases of breast cancer also appear to have defects in AMPK activation, although the mechanism is not known. We have found that AMPK is down-regulated in cells infected with hepatitis C virus (HCV). HCV may switch off AMPK to prevent its inhibitory effects on lipid and protein synthesis, required for viral replication. HCV infection activates the PKB/Akt pathway, leading to phosphorylation of the α 1 subunit of AMPK on Ser-485, thus inhibiting AMPK activation via phosphorylation at Thr-172. It is possible that this contributes to the elevated risk of hepatocellular carcinoma observed in humans with chronic HCV infection.

Finally, we have found that AMPK is directly activated by salicylate, and have evidence that at least some of the metabolic changes induced by the drug *in vivo* are mediated by AMPK. Salicylate is a natural product of plants whose medicinal use was described in ancient manuscripts. Its use has largely been replaced by derivatives such as salsalate and aspirin, but both are rapidly broken down to salicylate *in vivo*. It is possible that some of the beneficial effects of salicylate derivatives, including amelioration of insulin resistance and protection against colon cancer, might be mediated by AMPK.

011

Energy balance, metabolism and cancer prevention: mechanistic insights from transdisciplinary studies

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Introduction: The prevalence of obesity, an established risk factor for many cancers, has risen steadily for the past several decades in the US and many other countries. Unfortunately, the mechanisms underlying the obesity and cancer connection are not well understood, and new targets and strategies for offsetting the impact of obesity on cancer risk and/or progression are urgently needed.

Methods and results: We have established that calorie restriction (CR), the most commonly recommended dietary strategy for preventing or reversing obesity, inhibits spontaneous, transplanted and chemically induced tumors in a variety of animal models. In contrast, diet-induced obesity enhances tumorigenesis in many of these same models. We have also shown in a series of transgenic model systems and microarray studies that the insulin/insulin-like growth factor (IGF)-1 pathway appears central to many of the anti-cancer effects of CR and pro-cancer effects of obesity. Using AZIP/F1 transgenic mice (which lack white adipose tissue but have high levels of insulin, IGF-1 and inflammatory markers), and liver-specific IGF-1-deficient mice, we have reported that elevated IGF-1/insulin resistance/inflammation (which typically

accompany obesity), independent of the adipose tissue per se, appear to be the important targets for disrupting the obesity-cancer link. Also, genetic and pharmacologic (rapamycin) approaches suggest the Akt/mammalian target of rapamycin (mTOR) pathway (downstream of insulin and IGF-1 receptors) provides an important target for disrupting the obesity-cancer link.

Conclusion: A better understanding of the mechanisms underlying the energy balance-cancer link will facilitate the development of novel prevention and treatment strategies for offsetting the effects of obesity on cancer.

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O12

Excess body weight, diabetes and cancer: epidemiologic evidence implicating hormonal and metabolic mechanisms

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Epidemiological observations increasingly implicate nutritional energy balance as a key risk factor for cancer development. Excess body weight is associated with increased risks of cancers of the endometrium, breast (postmenopausal women), kidney (renal cell tumours), colon, pancreas and oesophagus (adenocarcinomas), and is also a well-documented risk factor for high-grade prostate cancer. By contrast, regular physical activity reduces the risks of breast and colorectal cancers and potentially other tumour types, and overall, excess weight and lack of physical activity have been estimated to potentially account for one quarter to half of the occurrence of the most frequent tumour types in affluent, industrialized societies. Animal experiments have shown uniformly protective effects of dietary energy restriction against tumor development.

Physiologic mechanisms that are thought to account for these effects of nutritional energy balance on cancer risks include changes in the metabolism of endogenous hormones, growth factors and inflammation factors, as well as in energy and nutrient status at the level of single cells. Together, these physiologic changes may stimulate cell growth and proliferation, inhibit apoptosis, and favour the occurrence of genetic mutations through increased oxidative stress. The key mechanisms that underlie these relationships of nutritional energy balance with cancer development may strongly depend, however, on tumour type.

Prospective cohort studies have shown relationships of risks of various cancer types with blood levels of glucose and insulin, and insulin-like growth factor-I. Furthermore, increased levels of circulating androgens and estrogens and reduced levels of progesterone are strongly implicated especially in the development of cancers of the endometrium and breast, among women with excess weight. The implication of specific hormonal or metabolic factors in cancer development is further increased by a growing body of evidence from human intervention studies – e.g. using selective estrogen receptor modulators [SERMs] and aromatase inhibitors – and epidemiological studies on the effects of specific hormonal medications (e.g., oral contraceptives and postmenopausal hormone replacement therapy; specific anti-diabetic drugs). Finally, several studies have shown increased risks of cancer, e.g. of the colorectum and endometrium, among subjects with higher than average serum levels of inflammation factors such as C-reactive protein, TNF-alpha or various interleukins.

According to present estimates, excess weight and lack of physical activity are likely the most important risk factors after smoking for cancer occurrence overall in western countries, and better knowledge of the true magnitude of the problem and of physiologic mechanisms involved can help define guidelines for primary prevention. At the same time, the knowledge gained about the hormonal and other physiologic mechanisms that link nutritional energy balance to cancer increasingly is also informing translational research into cancer preventive and curative treatments.

O13

Novel mechanisms by which fat cells regulate systemic insulin sensitivity and diabetes risk

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The adipose cell functions as an endocrine organ in addition to its role in energy storage. Adipocytes secrete hormones, cytokines and other factors that influence energy balance, glucose homeostasis, insulin sensitivity and vascular biology through effects in peripheral tissues and the central nervous system. In humans with obesity and type 2 diabetes, expression of the Glut4 glucose transporter is down-regulated selectively in adipocytes and this has systemic metabolic effects. Knocking out Glut4 selectively in adipocytes in mice causes insulin-resistant and increases the risk of diabetes while adipose-specific Glut4 overexpression confers enhanced glucose tolerance and insulin sensitivity in spite of increased adiposity. To discover novel pathways that regulate glucose homeostasis and insulin sensitivity, we used DNA microarray analysis of adipose tissue from mice with adipose-specific alterations in Glut4 expression. We found novel adipocyte-secreted proteins that alter systemic insulin sensitivity. In addition, gene set enrichment analysis showed coordinate regulation of lipogenic genes in adipose tissue. This led to the discovery that the glucose-responsive transcription factor, Carbohydrate responsive-element binding protein (ChREBP), is a dominant regulator of lipogenesis in adipose tissue and that its expression in adipose tissue is highly associated with insulin sensitivity in humans even in the presence of obesity. Furthermore, we identified a novel, potent ChREBP isoform and defined a new “feed forward” mode of regulation of ChREBP by which adipose-lipogenesis regulates systemic glucose homeostasis. This could provide new strategies for prevention and treatment of insulin resistance and type 2 diabetes.

O14

Up, down, and all around the epigenomic regulation of metabolism

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Nuclear receptors (NRs) transduce environmental and metabolic signals into alterations in gene expression by recruiting coregulators that alter chromatin structure. Rev-erbs alpha and beta (NR1D1 and NR1D2, respectively) are heme receptors that comprise negative limb of the circadian clock. The Rev-erbs function in the genome by recruiting complexes containing the nuclear receptor corepressor NCoR and histone deacetylase 3 (HDAC3). The histone modifying enzymatic activity of HDAC3 depends upon interaction with NCoR or SMRT, and disruption of this corepressor function alters circadian rhythms and metabolic physiology. In liver, circadian expression of Rev-erbs leads to their oscillating interaction with the genome. At specific times of day the Rev-erbs recruit NCoR and HDAC3 to thousand of genomic sites, with an enrichment for genes regulating lipid metabolism, and with a rhythm that is anti-phase to histone acetylation at these sites. The cistromes of Rev-erbs alpha and beta are nearly identical, and the coordinated activities of the Rev-erbs protect the clock and normal metabolic function. Thus Rev-erbs, NR corepressors, and HDAC3 team up to orchestrate a circadian epigenomic rhythm that plays a major role in the regulation of intermediary metabolism and nutrient storage in the liver. This pathway links the circadian environment to the epigenome, resulting in a daily re-routing of metabolites into lipid versus glucose metabolic pathways that is required for the normal physiological control of metabolism.

O15

Amplification of phosphoglycerate dehydrogenase diverts glycolytic flux and contributes to oncogenesis

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Most tumors display enhanced glucose metabolism compared to normal tissues [1-6]. The preferential conversion of glucose to lactate in cancer cells (the Warburg Effect) was one of the first known differences between tumor and normal cells and is believed to contribute to enhanced growth in tumor cells. However, the extent to which specific metabolic fluxes originating from glucose branch from central carbon metabolism and are utilized for anabolic processes is poorly understood. Here, we used an integrated, quantitative metabolomics approach combining NMR experiments with heavy isotope labeling and targeted mass-spectrometry. We carried out direct measurements of metabolic fluxes emanating from glucose metabolism [7].

We found that in some cancer cells, a relatively large amount of glycolytic carbon was diverted into serine and glycine biosynthesis. Serine can be synthesized from a glycolytic intermediate, 3-phosphoglycerate (3PG). 3PG is oxidized by an enzyme phosphoglycerate dehydrogenase (PHGDH) in an NAD-dependent manner to generate phosphohydroxypyruvate which is transaminated and then dephosphorylated to generate serine. Serine hydroxymethyltransferase then donates the side-chain of serine to the Folate pool to generate glycine.

We quantified the relative amount of glycolytic flux being diverted into serine and glycine biosynthesis and found that it was comparable to the amount of glucose passing through glycolysis and ultimately being converted to lactate. However, this large flux was observed to be present in only a subset of cultured cells. A non-tumorigenic breast epithelial cell line was found to have no detectable flux into serine and glycine biosynthesis. The observation that some but not all cancer cells divert a large amount of glycolytic flux into serine and glycine metabolism suggested that there might be a context in which this flux might be selected for in the development of cancer.

To search for this context, we investigated a pooled analysis of 3131 human cancers [8]. This analysis revealed that PHGDH, the gene that encodes the first enzyme in this biosynthetic pathway branching off of glycolysis is frequently present in a region of recurrent, localized copy number gain at genomic locus 1p12 ($q=1.18e-9$). The amplification was most commonly found in melanoma but was observed in other cancers such as esophageal adenocarcinoma and triplenegative breast cancer. We validated this finding in a collection of human melanoma tissue samples by comparing PHGDH expression in human tissues as measured by Immunohistochemistry with matched assessments of PHGDH copy number gain using fluorescence in situ hybridization. It was observed that PHGDH protein expression correlated with genomic copy number gain.

To assess whether cells containing the amplification were differentially sensitive to inhibition of PHGDH expression, we considered a panel of tumor-derived human Melanoma cell lines. Each melanoma cell line with PHGDH amplification exhibited significant flux into serine biosynthesis. We then carried out an RNA interference study and found that decreased PHGDH expression by RNA interference impaired growth in cell lines containing amplification of PHGDH and resulted in a distinct metabolic phenotype marked by accumulation of glycolytic intermediates.

PHGDH expression was also found to be associated with aggressive breast cancer subtypes. Since we observed no detectable flux in a non-tumorigenic breast epithelial cell line, we questioned whether enhanced PHGDH expression would have any phenotypic consequences.

We considered the effects of PHGDH in a model of breast tissue morphogenesis [9]. In this model, ectopic expression of PHGDH in MCF10A cells induced loss of cell polarity, disruptions in nuclear architecture and rescue of anoikis – each being phenotypic alterations

that predispose cells to tumorigenicity. Our findings demonstrate that altered metabolic flux stemming from glycolysis can be selected for in the development of cancer and contribute to cell transformation. Furthermore, our studies identify PHGDH as an attractive therapeutic target for subsets of human cancers.

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O16

Regulation of nutrient metabolism by nuclear receptor/FGF signaling pathways

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The regulation of metabolism in fed and fasted states is governed by hormonal and nutrient-derived signals that are mediated in part by nuclear receptors. Just as insulin and glucagon help the body store and mobilize energy through their membrane receptors, nutrient-derived lipids activate their cognate nuclear receptors (e.g., FXR and PPARs) to govern transcriptional programs involved in energy storage and utilization during times of nutrient excess and privation. Recent work has revealed that many of the actions of these nuclear receptors are mediated by two atypical fibroblast growth factors (FGF19 and FGF21) that function as endocrine-like hormones. The characterization of these pathways in normal and metabolic disease animal models has led to an understanding of their physiologic and pharmacologic mechanism of action.

O17

The histone deacetylase SIRT6, a critical modulator of metabolism and tumorigenesis

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Efficient glucose metabolism is critical for maintaining cellular viability. Under normal nutrient and oxygen conditions, glucose is converted to pyruvate, entering the mitochondria for oxidative phosphorylation and ATP production. Under hypoxia or nutrient stress, metabolism is switched to glycolysis, increasing lactate production and reducing mitochondrial respiration, a switch known to play an important role in cancer cells, as defined by Otto Warburg decades ago. Little is known whether chromatin plays a role in carbohydrate flux. The yeast Sir2 protein is an NAD-dependent histone deacetylase that senses the metabolic status of the cell and functions as a chromatin silencer to promote lifespan and genomic stability. Recently, we discovered that the mammalian SIRT6 is a chromatin factor that influences glucose

metabolism and DNA repair. In mice, SIRT6-deficiency provokes a profound and lethal hypoglycemia which culminates in accelerated death. At the cellular level, SIRT6 inactivation leads to increased cellular glucose uptake, higher lactate production and decreased mitochondrial activity. Our results indicate that SIRT6 directly regulates expression of several key glycolytic genes. In this context, SIRT6 co-represses Hif1 α , acting as a histone H3 lysine9 (H3K9) deacetylase to inhibit expression of glycolytic genes (Zhong et al, 2010). Strikingly, our new studies indicate that the “glycolytic switch” observed in the absence of SIRT6 provides a unique growth advantage in the context of tumorigenesis. Indeed, we find that SIRT6 deficiency causes transformation even in the absence of oncogenic signaling, and SIRT6 levels determine progression and disease-free survival in various human cancers. Our results suggest that SIRT6 might play a critical role in modulating the Warburg effect.

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O18

A transdisciplinary approach to obesity and cancer: from cells to society

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The increasing recognition of the complex, multidimensional relationship between excess adiposity and cancer risk, coupled with the high prevalence of obesity in the US, has motivated the scientific community to seek new research models and paradigms. Compared to traditional unidisciplinary research, transdisciplinary research is seen as having the potential to accelerate both discovery and its translation to practice, and eventually policy. In response, the National Cancer Institute (NCI) developed a centers grant mechanism in nutrition, energetics, and physical activity; referred to as the Transdisciplinary Research on Energetics and Cancer (TREC) Initiative (www.trecscience.org). These Centers foster collaboration among transdisciplinary teams of scientists with the goal of accelerating progress towards reducing cancer incidence, morbidity and mortality associated with obesity, low levels of physical activity and poor diet. This presentation will review key opportunities in transdisciplinary research, highlight lessons learned and note future challenges.

O19

Finding mechanisms from metabolic signatures of disease

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We seek to apply comprehensive metabolic analysis tools (sometimes called “metabolomics”) for understanding of mechanisms underlying chronic human diseases and conditions such as diabetes, obesity, and cardiovascular disease. Current approaches include analysis of metabolic flux by ¹³C NMR-based mass isotopomer analysis (in collaboration with Drs. Shawn Burgess and A. Dean Sherry and associates, Dallas, TX) and metabolic profiling of important groups of metabolic intermediates by both “targeted” and “unbiased” mass spectrometry (in collaboration with Drs. James Bain, Robert Stevens, Olga Ilkayeva, Brett Wenner, Michael Muehlbauer, Mark Butler, and David Millington at Duke). These tools have also been used to define mechanisms underlying development of peripheral insulin resistance in animals and humans. For example, we have recently identified perturbations of branched chain amino acid (BCAA) catabolism in

multiple cohorts of insulin resistant humans compared to normally insulin sensitive controls and have translated these findings to rodent models to demonstrate a contribution of BCAA to development of insulin resistance that is independent of body weight. In collaboration with Dr. Alan Attie at the University of Wisconsin, we have integrated transcriptomic and metabolomic analysis in mouse models to identify new pathways that control hepatic gluconeogenesis and PEPCK expression. Finally, with Svati Shah and Bill Kraus at Duke, we have identified novel metabolic signatures of imminent cardiovascular events, and are integrating genomic and metabolomics analyses in large cohorts of human subjects with a high incidence of coronary artery disease to identify pathways involved in metabolic variability and risk of cardiovascular disease. These examples will serve to illustrate the potential of comprehensive metabolic profiling methods for providing insights into metabolic disease mechanisms.

O20

Abstract not submitted for online publication

O21

Energy balance and cancer risk at the cellular and whole organism level: modification by metformin

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BMC Proceedings 2012, **6**(Suppl 3):O21

It has long been recognized that dietary restriction (DR) can inhibit carcinogenesis. Recent work has not only clarified the mechanisms involved, which involve the effects of DR on circulating hormones and cytokines, but also demonstrated molecular characteristics of tumors that determine the extent to which they are influenced by variation in host energy intake. Importantly, we and others have also demonstrated that a subset of tumors are growth-stimulated by excess caloric intake, and provided strong circumstantial evidence that hyperinsulinemia is one of the mediating factors. Metformin, a biguanide used in cancer treatment, has been associated with reduced cancer risk in some hypothesis-generating pharmaco-epidemiologic studies. Laboratory studies have provided evidence for some antineoplastic activity. However, this activity is confined to subpopulations including subjects who are obese and/or hyperinsulinemic. Laboratory models suggest that metformin may act in this context because it is well-known to reduce hyperinsulinemia as a consequence of its reduction of gluconeogenesis and hyperglycemia. At the cellular level, metformin acts to inhibit oxidative phosphorylation, and this may provide an additional mechanism that could operate in the subset of cancers or at-risk tissues that express the cell surface transport molecules required for metformin entry to cells. The ATP deficiency induced by metformin may have little effect on some cells, an AMPK-dependent cytostatic effect on others, and still others may suffer an energetic crisis and necrotic death. Current research is providing additional details and mechanistic details underlying these actions, defining the subsets patients for which biguanides may be useful in cancer prevention or treatment, and defining relevant doses for potential new indications. Investigating the possibility of ‘repurposing’ metformin for applications in oncology is ongoing as laboratory and translational research yield data that will help to optimize clinical trial design.

O22

Growth control and metabolism

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mTOR is the target of the immunosuppressive drug rapamycin and the central component of a nutrient- and hormone-sensitive

signaling pathway that regulates cell growth and proliferation. We now appreciate that this pathway becomes deregulated in many human cancers and has an important role in the control of metabolism and aging. We have identified two distinct mTOR-containing proteins complexes, one of which regulates growth through S6K and another that regulates cell survival through Akt. These complexes, mTORC1 and mTORC2, define both rapamycin-sensitive and insensitive branches of the mTOR pathway. I will discuss new results from our lab on the regulation and functions of the mTORC1 and mTORC2 pathways.

O23

Irisin and the therapeutic benefits of exercise

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BMC Proceedings 2012, 6(Suppl 3):O23

We are experiencing a worldwide epidemic of obesity and type II diabetes. Our group has been interested in the development of both white and brown fat, particularly at the level of gene transcription. PGC1 α was first described as a coactivator of PPAR γ in the control of brown fat-mediated thermogenesis. More recent work has shown that PGC1 α controls much of an exercise program in skeletal muscle. We have now found that PGC1 α expression and exercise control the expression of Fndc5, a membrane protein of skeletal muscle. Fndc5 is proteolyzed to give rise to a new secreted protein of 112 amino acids that we have called irisin. Irisin circulates in both mouse and man, and even mild elevations of irisin activates the browning of white fat, causing increased energy expenditure and reducing obesity. Irisin administration via adenoviral vectors also greatly improves glucose homeostasis in high-fat fed mice. Most recently we have identified cell surface binding of irisin that is likely to represent a cell surface receptor. These data indicate that irisin is a protein regulated in muscle by exercise that gives some of the benefits of exercise on energy homeostasis and metabolic disease.

O24

Does fructose play a role in metabolic disorders?

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There is evidence that high fructose diets can lead to the development of obesity, insulin resistance, and dyslipidemia in rodents model. In humans, however, the role of fructose in the recent increase in the prevalence of metabolic diseases remains much debated. Several epidemiological studies show a positive relationship between consumption of added sugar, fructose, or sweetened beverages on one hand, and metabolic disorders on the other hand, but fail to conclusively prove a causal relationship. Several short term overfeeding studies show that a high fructose diet can, over a short period of time, increase plasma triglyceride concentrations and in intra-hepatocellular lipid concentrations, stimulate hepatic de novo lipogenesis, modestly increase endogenous glucose production, and enhance post-glucose glycemic responses. Whether these studies are relevant to the pathophysiology of the metabolic syndrome is however not known, and several important issues remain however to be addressed. More specifically, whether consumption of fructose causes metabolic disorders when consumed as part of an energy-balanced diet is not known. The interactions between fructose intake and other environmental factors such as other macronutrients or physical activity have also not been addressed. This presentation will summarize the present knowledge regarding the metabolic effects of short term high fructose diets in humans. Novel data will be presented to support that a high fructose diet increases plasma triglycerides independently of energy balance. Finally, the influence of other environmental factors (dietary protein intake, exercise) on the metabolic effects of fructose will be presented.

O25

Beyond insulin: rethinking cellular metabolism

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BMC Proceedings 2012, 6(Suppl 3):O25

The latest cancer therapeutics are almost all inhibitors of oncogenes or their downstream mediators. With the exception of imatinib, most such drugs have been disappointing in the clinic. In contrast, existing successful cancer drugs have a therapeutic index because they render the consequences of oncogene activation selectively toxic to the cancer cell. A renewed focus on this type of approach appears warranted and should be considered.

O26

MCT4 is an important determinant for the growth of highly glycolytic and aggressive malignancies

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BMC Proceedings 2012, 6(Suppl 3):O26

Constitutive upregulation of glycolysis is likely to be an adaptation to hypoxia that develops as premalignant lesions grow progressively distant from their blood supply. Increased lactic acid production caused by the upregulation of glycolysis results in microenvironmental acidosis. Cell populations that emerge from this adaptation have a powerful growth advantage, as they alter their environment through increased glycolysis in a way that is toxic to other phenotypes, but harmless to themselves or their re-programmed stromal environment. Our goal is to elucidate the molecular basis for this aberrant behavior, and harness that knowledge towards the development of effective therapeutic strategies against malignant tumors. Here we describe *in vitro*/*in vivo* functional analysis of MCT1, MCT4 and CD147 in three different cell contexts, where MCT1 and MCT4 are expressed either alone or together and confer differential growth advantages under hypoxic or normoxic conditions. We show the selective enrichment of CD147 and MCT4 in highly tumorigenic CD133/CD44 positive cells from colon cancer patient-derived xenografts.

Our findings indicate that MCT4 is an important determinant for the growth of highly glycolytic and aggressive malignancies, which so far has not been explored as a target for therapeutic purposes and may provide a unique avenue for anticancer therapy. We will provide *in vitro* and *in vivo* evidence for tumor contexts, where the selective inhibition of MCT4 is sufficient as well as contexts, for which the combined inhibition of MCT1 and MCT4 is required for providing a therapeutic benefit.

O27

Carbohydrate restriction uniquely benefits metabolic syndrome and saturated fat metabolism

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BMC Proceedings 2012, 6(Suppl 3):O27

Metabolic syndrome (insulin resistance syndrome) represents a group of physiologic signs that indicate a predisposition to obesity, diabetes, and cardiovascular disease. Consistent with the idea that intolerance to carbohydrate is an underlying feature of metabolic syndrome, we present results showing that a low carbohydrate diet results in global improvement in traditional and emerging markers associated with metabolic syndrome. Control diets, restricted in fat, are shown to be less effective. Recent research results mandate a careful re-evaluation of the widespread belief that dietary saturated fat is harmful. Specifically, multiple recent reports find no association between dietary saturated fat intakes and cardiovascular disease (CVD). There is, however, a consistent pattern of increased risk for both CVD and type-2 diabetes associated with increased levels of saturated fatty acids (SFA) in circulating lipids. This raises the important question as to what

contributes to increased levels of saturated fat in the blood? Whereas dietary intake of saturated fats and serum levels of SFA show virtually no correlation, an increased intake of carbohydrate is associated with higher levels of circulating SFA. This leads to the paradoxical conclusion that dietary saturated fat is not the problem; rather it's the over-consumption of carbohydrate relative to the individual's ability to metabolize glucose without resorting to de novo lipogenesis. From this perspective, insulin resistant states like metabolic syndrome and type-2 diabetes can be viewed as carbohydrate intolerance, in which a high carbohydrate intake translates to increased serum SFA and therefore increased risk. We all stand to benefit, both now and in the future, if a well-formulated low carbohydrate diet becomes an accepted option in promoting health across many sub-groups in our population.

POSTER PRESENTATIONS

P1

Association of obesity with down-regulation of heat shock protein 40 expression and evidence that exercise retrieves its normal expression

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BMC Proceedings 2012, 6(Suppl 3):P1

Background: Obesity is a major risk factor for insulin resistance and diabetes. It is a metabolic disorder characterized by chronic-low grade inflammation and aberrant regulation of various forms of stress response in key metabolic sites. Heat shock response; a crucial host-defense mechanism against stressful conditions, is one of the key pathways that was shown to be deregulated in obesity-induced insulin resistance. Patients with T2D have reduced gene expression of Heat shock proteins (Hsps) which correlates with increased insulin resistance. Consistent with this, therapies that induce heat shock response such as heat therapy or physical exercise are associated with beneficial outcome. The objectives of this study are: 1) Investigate the expression pattern of Hsps and other chaperones and stress related proteins between lean and obese subjects, 2) Evaluate the effect of a defined exercise protocol on their expression pattern and 3) Correlate their expression with the inflammatory, metabolic and oxidative stress biomarkers.

Materials and methods: A total of 600 human subjects divided into 3 equal groups using the body mass index as a reference (lean, overweight and obese) are being enrolled in a defined exercise protocol for 6 months. Blood samples and abdominal subcutaneous adipose biopsies were collected at 0, 3 and 6 months of the exercise. Gene expression profile was done by RT-PCR using RT-profiler-heat shock protein array kit consisting of 84 heat shock related genes. Cytokine and metabolic markers were investigated by multiplexing technology (Bioplex) using plasma samples. Markers of oxidative stress, namely ROS, and TBARS were measured on plasma or serum using commercially available kits. Western blotting and immunohistochemistry techniques were used to validate the gene expression data.

Results: The analysis carried out on a first set of samples indicated differential expression of DNAJC5B, a member of Hsp-40 and crystallin alpha-B (CRYAB) genes between the lean and obese subjects. In obese subjects, we observed an upregulation (3.2 folds) of CRYAB gene expression. By contrast, the expression of DNAJC5B was down-regulated (>2 folds) in obese subjects in comparison to lean subjects. Interestingly, within 3 months of regular exercise, the levels of DNAJC5B in obese subjects were comparable to the lean subjects, suggesting that exercise can antagonize obesity-mediated DNAJC5B repression. The downregulation of DNAJC5B in obese subjects is associated with an increase in the inflammatory response such as TNF- α , IP-10 and MIP-1B. We also found a negative correlation between DNAJC5B expression and the release of metabolic and oxidative stress markers such as leptin, insulin, visfatin, ROS and TBARS.

Conclusions: Our result demonstrated the differential expression of CRYAB and DNAJC5B genes in obese subjects in comparison to lean and highlighted their roles in obesity. Exercise was able to restore the aberrant expression of DNAJC5B gene and the existence of a negative correlation between DNAJC5B expression and the inflammatory, metabolic and stress responses is suggestive of a protective role of DNAJC5B and could therefore be a potential therapeutic target.

P2

Alcoholic liver steatosis in mice is aggravated by low-protein diet and reversed by FXR agonist

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Background: Hepatic steatosis refers to the accumulation of triglycerides in hepatocytes, and it can be attributed to excessive ethanol consumption. The liver is the main organ of ethanol biotransformation and therefore, it can suffer with oxidative stress generated by ethanol. Since the FXR agonist 6ECDCA regulates adipose cell function, the aim of this work is to evaluate the participation of oxidative stress in ethanol-induced liver lesions and test the effects of FXR agonist against alcoholic liver steatosis development. For thus, diets with different amount of protein were used.

Material and methods: Swiss male mice (8-10 weeks) were separated in 2 groups (n=24), which received liquid diet containing 10% ethanol or water (control group) for 6 weeks, as well as a low-protein diet (6%) or norm-protein diet (23%). In the last 15 days of the diet, mice that received ethanol or water were separated again for oral treatment, performing 8 groups (n=6) in the total. From these groups, 4 received FXR agonist 6ECDCA (3 mg.kg⁻¹) and 4 received 1% tween (vehicle). Following this treatment, animals were anesthetized for sample collections (hepatic tissues and blood), in order to perform: serum biochemistry assays [aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol and triglycerides], hepatic oxidative stress (catalase, superoxide dismutase, glutathione-S-transferase, reduced glutathione and lipid peroxidation), liver histology (hematoxylin-eosin, Sudan black and Nile blue staining) and gene expression of *Srebp1f*, *FAS*, *PPAR α* , *CYP7a1*, *HMGCoA reductase*, *ApoB*, *Scd1*, *p53* and *Bax*.

Results: Ethanol associated with low-protein diet (6%) induced hepatic oxidative stress, increased plasmatic ALT and AST, and induced hepatic lipid accumulation. Many of these parameters were reverted by administration of 6ECDCA, including significant reduction in hepatic steatosis and improvement of antioxidant enzymes. These effects are possibly mediated by regulation of the genes *Srebp1f* and *FAS*, since both had the expression reduced by the FXR agonist.

Conclusion: Ethanol induced intense hepatic steatosis when used in combination with low-protein diet (6%). Diet with regular amount of protein (23%) seems to prevent the hepatic effects of alcohol. Evaluating the participation of oxidative stress and FXR in the pathogenesis of alcoholic fatty liver disease in mice we demonstrated that 6ECDCA reverses the accumulation of lipids in the liver and decreases the hepatic oxidative stress. Thus, we speculate a possible therapeutic action of FXR agonists in alcoholic liver disease aiming to prevent the progression of this disease to more severe stages such as fibrosis, cirrhosis and hepatocellular carcinoma.

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P3

Abstract not submitted for online publication

P4

Expression of the glucose transporters GLUT1, GLUT3, GLUT4 and GLUT12 in human cancer cells

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BMC Proceedings 2012, 6(Suppl 3):P4

Background: It is well known that cancer cells display increased glucose uptake and consumption [1]. In a rate-limiting step for glucose metabolism, the glucose transporter (GLUT) proteins facilitate glucose uptake across the plasma membrane. In human cells, 14 different GLUT (1-14) isoforms have been discovered. Of these, GLUT1, 3 and 4 have been extensively studied while the more recently discovered GLUT12 has high relevance to cancer [2]. A comprehensive view of the basal levels of GLUT proteins in cancer is lacking and the exact role of these proteins in cancer cell metabolism is unclear. The present study examined GLUT expression in a number of human cancer cell lines.

Materials and methods: Real-time PCR was used to quantify mRNA levels of GLUT1, GLUT3, GLUT4 and GLUT12 in lung (A549, NCI-H460, SK-MES-1, H1299), breast (MDA-MB-231, MCF-7), and prostate (PC-3, 22RV1, LNCaP) cancer cells and the normal epithelial cells MRC-5, 184B5 and PNT1A. Protein expression of the glucose transporters was examined by immunoblotting and fluorescence microscopy. The expression of GLUT1 and GLUT3 was immunohistochemically examined in tumors from Balb/c nude mice xenografted with A549, H1299 and PC-3 human cancer cells.

Results: GLUT1, GLUT3, GLUT4 and GLUT12 mRNA was detected at various levels in all cells examined. Cancer cells were found to have significantly higher expression of GLUT proteins than the corresponding normal epithelial cells at both the mRNA and protein level. Furthermore, GLUT expression in cancer was found to follow an abnormal expression pattern compared to healthy cells. GLUT1 and GLUT3 were detected in vivo in human cell line xenograft tumors.

Conclusions: These findings describe GLUT expression in a number of cancer cells with different mutations and histologies. The differences in the levels and patterns of glucose transporter expression between normal and cancerous human epithelial cells suggests GLUT proteins may serve as an attractive target for the development of therapeutic agents in lung, breast and prostate cancer.

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P5

Phytochemical profiling of strawberry fruits, and bioactive compounds from the same selected cultivar in human plasma during a medium-term consumption study

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Background: It is known that a common denominator in the pathogenesis of most chronic diseases is the involvement of oxidative stress, related to the production by all aerobic organisms of reactive oxygen species and reactive nitrogen species. Also the modern and more complex theories on the role of oxidative stress in biological processes confirms the importance of a balanced equilibrium between oxidant production and antioxidant defenses, for preserving health and longevity. In parallel, a growing body of epidemiological studies suggests a consistent association between the consumption of plant-based diets and a lower incidence of several chronic pathologies, including cancer, cardiovascular and neurodegenerative diseases.

Materials and methods: The first aim of this work was to evaluate the nutritional quality of the strawberry cultivar *Sveva*, by measuring the total antioxidant capacity, total phenolic, total flavonoid, and total anthocyanin contents of the strawberry extract. In addition, the vitamin C content was quantified by HPLC, while anthocyanin pigments were identified using HPLC-DAD-ESI/MS.

The second aim of this work was to assess the effects of strawberry consumption on biomarkers of antioxidant status, in healthy subjects. A pilot study was carried out, and the 24 screened participants were involved in a medium-term strawberry consumption test. They were asked to consume 300 g of strawberry per day for 15 days, preferably at mid-morning and mid-afternoon between meals. The potential changes in plasma markers of antioxidant status were evaluated, by measuring the strawberry-dependent variation in plasma total antioxidants and in serum concentrations of Vitamin C and Acid uric by HPLC with electrochemical detection. Moreover, plasma protein carbonyl content, the most commonly used marker of protein oxidation, and plasma reactive oxygen metabolites (dROMs), one of the most used marker of hydroperoxides levels, were evaluated. Statistical analyses were performed using STATISTICA software and the data were analyzed with the Wilcoxon test. Results are expressed as mean values $\pm$ standard error. Differences at $p < 0.05$ were considered statistically significant.

Results: The cultivar *Sveva* presented high nutritional attributes, especially regarding total phenolic, total flavonoid and vitamin C contents, and showed relevant and well-balanced antioxidant, micronutrient and phytochemical composition. In addition, the medium-term intake of strawberries resulted in a significant increase in plasma antioxidant capacity measured by TEAC, FRAP and BAP assays in all subjects, independently of the individual baseline levels. Finally, significant increases in ascorbate, but not in urate, concentrations were observed in serum, as well as a significant decrease in protein carbonyl content and in dROMs in plasma.

Conclusions: Dietary antioxidants from fruit seem to play an important beneficial role in improving antioxidant defenses of the human body against the development of chronic diseases [1], so that the availability of high quality and nutritionally enriched fruit may be a useful tool when planning healthy diets. Strawberries contain many important dietary components including vitamins and minerals, and are a rich source of phytochemical compounds, which seem to have relevant biological activity on human health [1].

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P6

Myc-Foxo, a link between glutaminolysis and autophagy

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BMC Proceedings 2012, 6(Suppl 3):P6

Our recent observations that dMyc expression in the *Drosophila* FB - a metabolic tissue with similar physiological functions as mammalian adipose tissue and liver - remotely influences the development of the whole animal, allows us to explore its function in a living growing organism. dMyc in FB increases animal survival, which correlates with an increased level of autophagy, especially visible in FB from animals under starvation. Our preliminary data show that in addition to enzymes responsible for glycolysis, dMyc upregulates in FB the expression of the putative glutamine transporter slcA7/minidiscs and of glutaminase, the enzyme that converts glutamine to alpha-ketoglutarate with the production of ammonia. By varying the nutrient availability, we found that dMyc expression induces the accumulation of high levels of ammonia in the supernatant of S2 cells. Ex-vivo cultures of FB incubated in these supernatants exhibited autophagy, suggesting that dMyc is responsible for the expression of soluble pro-autophagic factors, possibly ammonia. In FB autophagy is particularly evident during starvation, conditions where dFOXO transcription sustains dMyc expression. Using genetic manipulation of components of glutamine metabolism we are currently exploring if the relationship between dMyc and dFOXO in FB affects ammonia production and

autophagy via glutamine catabolism. With these experiments we aim to understand whether FOXO regulates Myc to induce glutamine signaling, placing FOXO-function relevant for glutaminolysis, which is one of the most relevant survival pathways in tumor cells.

P7

Myostatin inhibition improves hyperglycemia and hyperphagia in lipodystrophic mice

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Background: Skeletal muscle is a key peripheral metabolic tissue: Muscle is responsible for most insulin-stimulated glucose uptake, and muscle insulin resistance is the first defect detectable in the development of type 2 diabetes. Recent studies show that muscle mass is inversely correlated with insulin resistance in humans. The growth factor myostatin (MSTN) is a negative regulator of skeletal muscle size. *Mstn* KO mice or mice expressing a dominant negative activin type receptor type IIB (Acrv2B) specifically in muscle (Muscle-DN mice) have increased muscle mass, reduced adipose mass and improved insulin sensitivity. The improvements in insulin sensitivity could be due to the direct effects of the loss of MSTN in muscle or to the secondary effects of reduced adiposity or both. Our hypothesis is that MSTN inhibition specifically in the muscle can improve hyperglycemia in diabetic mice independent of changes in adiposity. To test this hypothesis, we inhibited MSTN signaling in a diabetic model of generalized lipodystrophy (A-ZIP/F-1 mouse model) to analyze its effects on glucose metabolism separate from effects on adipose mass. The A-ZIP/F-1 fatless mouse is a model of lipodystrophy characterized by the lack of white adipose tissue, diabetes, ectopic lipid accumulation and hyperphagia.

Materials and methods: MSTN signaling was inhibited in A-ZIP/F-1 mice by crossing them to Muscle-DN mice or by injection of soluble Acrv2B. Blood glucose, insulin, insulin sensitivity, triglyceride levels, and food intake were analyzed. The effects of blood glucose on food intake were examined in A-ZIP/F-1 mice by administration of Phloridzin.

Results: The development of hyperglycemia, hyperinsulinemia and lipidemia in A-ZIP/F-1 mice was completely suppressed by blocking MSTN function in muscle. Blocking MSTN in A-ZIP/F-1 mice also prevented hyperphagia without any apparent increase in the adipokine leptin. Blood glucose and food intake were also reduced by pharmacologic MSTN inhibition in diabetic A-ZIP/F-1 mice using a soluble receptor. Decreasing blood glucose in A-ZIP/F-1 mice by administration of Phloridzin did not alter food intake.

Conclusions: These results show that MSTN inhibition can ameliorate diabetes in mice and that this response does not depend on reducing adipose mass. Furthermore, because energy intake is regulated by the central nervous system, our results suggest that muscle may send an as yet unknown signal to the brain, whether directly or indirectly, to regulate energy intake.

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P8

Impact of stromal microenvironment on metabolic phenotypes in breast cancer: evidence for stroma-influenced Warburg effect

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BMC Proceedings 2012, 6(Suppl 3):P8

Background: Cancer cells have altered metabolism, with an increased rate of glucose uptake, increased glycolysis, and due to the Warburg phenomenon, increased production of biomass (amino acids and

nucleic acids) as metabolic byproducts. Association of these metabolic changes with breast cancer subtype and tumor gene expression has not been evaluated, nor is it well understood what biological processes and characteristics are most important in driving altered metabolic state in breast cancer.

Experimental design: Thirty-four breast tumors and six samples of cancer-adjacent normal tissue were analyzed by liquid and gas chromatography (LC/GC) mass spectrometry (MS) to determine the expression of 379 metabolites. The same tissues were also used to perform gene expression microarrays and the resulting gene expression profiles were used to identify tumor subtype. These data combined with clinical tumor characteristics were evaluated in association with metabolic phenotype. To complement these analyses of human tissues, in vitro assays using human breast cancer cells alone and in coculture with cancer-associated fibroblasts (CAFs) were performed to study glucose uptake and utilization.

Results: Unsupervised cluster analysis on all metabolites resulted in two main clusters, with differences in the prevalence of aggressive tumor subtypes in each cluster. Characteristic of the Warburg phenomenon, four groups of metabolites showed differential levels between the two tumor clusters: amino acids, sugars, nucleic acids, and citric acid (TCA) cycle metabolites. Normal breast tissue samples were similar to less aggressive tumors with low biomass production and decreased levels of glycolysis/TCA byproducts. Parallel to these results in human tissues, glucose uptake was increased in basal-like breast cancer cells when they were cocultured with fibroblasts, but less aggressive luminal-fibroblast cultures did not show increased glucose uptake. Rather, in luminal cocultures, the fibroblasts appeared to suppress glucose uptake. Glucose utilization (oxidation and glycogen synthesis) was also higher in basal-like mono- and cocultures compared to luminal cultures. Consistent with these findings that the stroma influences metabolic phenotype, tumors that had active wound response gene expression had the more aggressive, Warburg-like metabolic profile.

Conclusions: We observed distinct metabolic profiles associated with more aggressive breast cancer tumors. Based both on the observed patterns of glucose uptake and utilization in cocultures, and on the association between wound response activation and metabolic profile, these metabolic signatures appear to be influenced by multiple factors, including the stroma. These data suggest that integration of genomic and metabolic data provide insights for better understanding a Warburg effect in human breast cancer that may be regulated by the stroma.

P9

Dysregulated metabolism and the regulation of aromatase in breast adipose stromal cells in obesity and cancer

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Background: The risk of breast cancer in postmenopausal women is increased two-fold with obesity, and the majority of breast tumours that arise are oestrogen-dependent. After menopause, when the ovaries cease to produce oestrogens, it is the local production of oestrogens within the breast adipose stromal cells (ASCs) which promotes and sustains tumour growth. This is largely due to the increased expression of aromatase, responsible for the conversion of androgens to oestrogens. Aromatase expression in breast cancer is known to be under the control of a proximal promoter, promoter PII, which is maximally activated by cAMP-dependent mechanisms. We have previously demonstrated that the LKB1/AMPK pathway is a key negative regulator of aromatase expression within the breast by inhibiting the nuclear translocation of the CREB co-activator CRTC2 [1]. We have also demonstrated that the tumour-derived factor prostaglandin E2 (PGE2) and the obesity-associated factor leptin stimulate aromatase expression by inhibiting LKB1 and AMPK expression and activity. Hypoxia inducible factor-1α

(HIF1 α) is emerging as a potent regulator of glycolysis in tumour cells and we have identified a putative hypoxia response element in aromatase promoter PII immediately adjacent the cAMP response element, known to be bound by the CREB-CRTC2 complex in ASCs in breast cancer. We therefore hypothesise that HIF1 α may be involved in regulating aromatase in breast cancer.

Materials and methods: Primary human breast ASCs were isolated from tissue after breast reduction surgery. Real-time PCR, Western blotting, immunofluorescence and high content screening were used to assess HIF1 α expression and localisation after PGE2 treatment. Chromatin immunoprecipitation (ChIP) was performed to examine the interaction of HIF1 α with aromatase promoter PII and reporter assays were performed to assess the effect of HIF1 α on PII activity. Double immunohistochemistry for HIF1 α and aromatase was also performed on sections of formalin-fixed paraffin-embedded breast tissue from breast cancer patients and cancer-free patients.

Results: We have found that PGE2 increases HIF1 α transcript expression, nuclear localisation and binding to aromatase PII in primary human breast ASCs. Moreover, HIF1 α causes a significant increase in PII activity and acts cooperatively with CREB to cause this induction. Data from breast cancer patient samples demonstrates that HIF1 α is also increased in tumour-associated ASCs compared to breast tissue from cancer-free women. Interestingly, the majority of ASCs from breast cancer patient samples display staining for both HIF1 α and aromatase.

Conclusions: This study is part of a growing body of evidence indicating that dysregulated metabolism is not only a characteristic of adipocytes in obesity and epithelial cells in cancer, but also occurs in tumour-associated ASCs. We demonstrate that dysregulation of metabolic pathways is accompanied by an increase in aromatase expression within the breast adipose and provide an additional mechanism whereby obesity is linked to breast cancer. Clinical studies are currently underway to explore the use of drugs which target these pathways, such as metformin, for their use as novel aromatase inhibitors for the prevention and treatment of postmenopausal breast cancer.

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P10

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P11

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P12

The *in vivo* function of the p53 target gene TIGAR

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The p53 tumour suppressor inhibits tumour development via various mechanisms such as apoptosis, inhibition of proliferation or the activation of senescence. Recently, several studies have indicated a novel role of p53 in the regulation of energy metabolism. Previously we have discovered TIGAR, a p53 target gene that acts as a fructose-2,6-bisphosphatase. TIGAR therefore can redirect glucose from the glycolytic pathway to the pentose phosphate pathway (PPP), which promotes NADPH production to generate reduced glutathione for protecting against ROS, and also ribose 5 phosphate production for nucleotide synthesis. In order to understand the function of TIGAR *in vivo*, we generated TIGAR deficient mice. We have determined a

critical role of TIGAR in rapidly proliferating tissue, either for repair after damage or during tumor development.

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P13

Lack of aminoacids in mouse hepatocytes in culture induces the selection of preneoplastic cells

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Protein malnutrition occurs when there is insufficient protein to meet metabolic demands. Previous works have indicated that cycles of protein fasting/refeeding enhance the incidence of early lesions during chemical carcinogenesis in rat liver. In mammals, protein malnutrition causes high susceptibility to infection diseases and cancer. In liver, both caloric and proteic malnutrition cause severe metabolic changes [1].

The general objective of this work was to study the effect of aminoacids (Aa) deprivation on the proliferation and survival of hepatocytes, to understand its possible involvement in the generation of pre-neoplastic stages in the liver.

Experimental procedures: cell line derived from newborn mice hepatocytes (Parental cells=Par) [2] were cultured in complete medium, or aminoacid-starved medium (PM=private medium).

When hepatocytes are cultured in the absence of Aa, the cells detach and die through apoptosis. After 72 h of culture with PM the few surviving cells were incubated with complete medium. After changing the medium, cells began to proliferate and expanded (Selected Cells=Sel).

Sel cells showed a significant higher proliferation rate than the Par ones. This conclusion was confirmed by studying the incorporation of [3H]-Thymidine as an analysis of DNA synthesis. In the presence of different concentrations of Fetal Bovine Serum, indicating that response to extracellular mitogens is enhanced in these cells. The response to the Transforming Growth Factor-beta (TGF- β), a physiological inducer of hepatocyte apoptosis whose concentration is elevated in liver tumors to counteract the abnormal growth of preneoplastic cells [3-5], is altered in Sel cells. Both TGF- β -induced decrease in cell viability and caspase-3 activation were attenuated in Sel when compared to the parental cells.

Both p-ERKs and p-AKT levels were much higher in Sel cells than in the Par ones. Downstream AKT signals, such as p70S6 and GSK3 were also higher phosphorylated in Sel cells. Interestingly, showed increased levels of p-SRC, which correlated with increase in the levels of phosphorylation of the EGF receptor (EGFR). These results indicate that the EGFR/SRC pathway might be overactivated in Sel cells. Sel cells expressed higher levels of EGFR ligands, such as TGF- α and HB-EGF; semiquantitative RT-PCR analysis clearly revealed that both ligands were overexpressed in Sel cells, when compared with normal hepatocytes. Moreover it is noteworthy that MAPK proteins c-JNK and p38, which remained increased in Sel cells.

In conclusion, results presented indicate that it is possible to isolate *in vitro* a population of hepatocytes that are able to survive in the absence of Aa, which has higher capacity to proliferate, showing a preneoplastic phenotype. This could explain why the alternately deprivation of proteins in diet could induce hepatocarcinogenesis susceptibility.

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P14

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P15

Enhanced cardiac fatty acid utilization induced by high dietary fat: a potential regulatory role for mitochondrial aconitase

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BMC Proceedings 2012, 6(Suppl 3):P15

Background: Obesity is an independent risk factor for cardiovascular disease and Type 2 diabetes, a condition that enhances the risk for various cardiomyopathies. Obesity is known to induce a state of metabolic inflexibility within the heart characterized by increased mitochondrial reliance on fatty acids for energy production. While the molecular mechanisms are not fully clarified, these changes in metabolism are thought to contribute to degeneration of cardiovascular function. The focus of my research is to investigate regulatory mechanisms that control diet-induced changes in fatty acid utilization (β -oxidation) by cardiac mitochondria.

Materials and methods: In the current study, we used quantitative proteomics, mitochondrial respiratory analysis, and enzymology to investigate factors that contribute to diet-induced changes in fatty acid utilization by cardiac mitochondria (β -oxidation). We employed C57B/6J mice fed a high fat diet (60% kcal from fat) as a model of obesity with mice fed low a fat diet (10% kcal from fat) serving as controls.

Results: Whole pathway analysis by mass spectrometry revealed elevated expression of β -oxidation enzymes with no change in Krebs cycle enzymes within 2 wk on the high fat diet up to ≤ 60 wk. These observations indicate a mismatch between β -oxidation and the Krebs cycle that is responsible for the consumption of β -oxidation product, acetyl CoA. This could lead to the buildup of cardiotoxic fatty acid derived intermediates. Subsequent, enzymatic analysis revealed that citrate synthase, isocitrate dehydrogenase, and α -ketoglutarate dehydrogenase, the key regulatory enzymes of the Krebs cycle, exhibited no change in activity. However, mitochondrial aconitase activity was increased approximately 60% with no change in expression suggesting a role for post translational modifications. Increased aconitase specific activity was observed within 2 wk on the high-fat diet and persisted with extended dietary durations (≤ 60 wk).

Conclusions: The increase in cardiac mitochondrial aconitase activity in response to high dietary fat highlights a potential novel regulatory role for the enzyme in β -oxidation. In the cytosol, citrate can be converted to malonyl CoA, a potent inhibitor of β -oxidation. Citrate is also an allosteric activator of acetyl CoA carboxylase, the enzyme that produces malonyl CoA. Since aconitase converts citrate to isocitrate, an increase in aconitase activity within the mitochondria has the potential to reduce citrate efflux and thus promote β -oxidation. These events could contribute to dietary-induced metabolic inflexibility.

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P16

Loss of E2F compromises mitochondrial function and protects cells from irradiation-induced apoptosis in *Drosophila*

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Background: In *Drosophila*, an activator, dE2f1, acts in parallel to p53 to trigger apoptosis in response to a cellular stress such as irradiation. It has been previously reported that inactivation of dDP, a heterodimeric partner of dE2f1, fully blocks irradiation-induced apoptosis in the eye imaginal disc.

Materials and methods: To understand the basis of this phenomenon, we have examined the effect of a dDP mutation on irradiation induced transcriptional program by using gene expression microarrays.

Results: Surprisingly, gene expression profiling revealed that the normal transcription program induced in response to irradiation in wild-type animals was also properly induced in dDP mutant animals. However, we found that the loss of dDP resulted in down-regulation of expression of a large panel of mitochondria-related genes. ChIP-seq and ChIP confirmed that many of these genes are indeed direct dDP targets. Consistently, mitochondrial function was severely compromised in dDP mutant eye discs. Down-regulation of several of mitochondria-related dDP target genes mimicked the dDP mutant phenotype and was sufficient to protect the eye discs from irradiation-induced apoptosis.

Conclusions: We concluded that the lack of irradiation-induced apoptosis in dDP mutants is not a consequence of the failure to induce the normal apoptotic response, but rather the result of a separate dDP/dE2F dependent transcriptional program regulating expression of genes involved in mitochondrial biogenesis. Thus, our data uncover a previously unappreciated role of dE2F/dDP in direct regulation of mitochondrial function and identify the particular settings when such regulation becomes important in vivo.

P17

Visfatin and RBP4 gene expression levels in different adipose tissues and insulin resistance

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Background: As an endocrine organ, adipose tissue produces and secretes lots of hormones and adipokines that play important roles in metabolism and inflammation [1]. Varied adipose tissues may affect the inflammation distinctively, mainly because of the different secretion levels of the adipokines. Adipokines like visfatin and retinol binding protein-4 (RBP4) may have important roles in insulin sensitivity and glucose uptake to the cell [2, 3]. Our research focuses on different tissue secretions of visfatin and RBP4 and their inflammatory properties.

Materials and methods: Potential subjects were informed of the voluntary nature of the study and asked to sign a consent form to participate in the study. Fifteen morbidly obese (BMI ≥ 40) subjects participated in the study. All patients underwent Roux-en-Y gastric bypass surgery. The subjects had their blood drawn as per the usual treatment protocol before surgery. The subjects also had the following tissue samples taken at the time of the surgery: subcutaneous adipose tissue, omental adipose tissue, and mesenteric adipose tissue. These tissues were immediately washed in Krebs-Ringer phosphate solution and frozen in liquid nitrogen in the operation room. Total RNA was isolated using Qiagen RNeasy Lipid Kit (Qiagen, Valencia, CA) and cDNA was synthesized from RNA using SuperScript III protocol (Life Technologies, Carlsbad, CA). Real-time quantitative PCR was used to quantify relative gene expression of Visfatin and RBP4. Results were analyzed using Student's t test with P < 0.05 being significant.

Results: Visfatin level was significantly higher in omental adipose tissue than mesenteric and subcutaneous adipose tissues (p < 0.05). There was not any significant difference for visfatin levels between mesenteric and subcutaneous adipose tissue. RBP4 was higher in subcutaneous adipose tissue (p < 0.05) and there was not any significant difference between mesenteric and omental adipose tissue. There was not any significant relationship between blood glucose and HbA1c levels and visfatin and RBP4 tissue levels.

Conclusions: Although both the omental and mesenteric adipose tissues are abdominal fat tissues, they have significantly different visfatin expression levels. The relationship between these adipokines and insulin resistance can be better studied with a larger sample size.

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P18

The role of SIRT3 in regulating cancer cell metabolism

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Background: Sirtuins are a family of NAD⁺-dependent deacetylase, deacylase, and/or mono-ADP ribosyltransferase enzymes involved in regulation of many biological processes. Mammals contain seven sirtuins, three of which are localized to the mitochondria (SIRT3-5). SIRT3 has been shown to be the major mitochondrial deacetylase that regulates metabolic enzymes and promotes oxidative metabolism and energy production. Loss of one copy of the SIRT3 gene is observed in various human cancers. Thus, we examined the role of SIRT3 in regulating metabolism in cancer cells.

Materials and methods: We have examined glucose uptake and lactate production in a variety of human cancer cell lines, as well as wild-type and SIRT3 null mouse embryonic fibroblasts (MEFs). In addition, we have utilized steady-state metabolomics to determine the metabolic profile of wild-type and SIRT3 null MEFs. Lastly, we utilized a combination of techniques, including quantitative RT-PCR and Western blotting, to examine the mechanism by which SIRT3 regulates cancer cell metabolism.

Results: Our data show that loss of SIRT3 increases glucose uptake and lactate production. Based on these and previous results from our laboratory, SIRT3 functions as a tumor suppressor to repress the Warburg effect by decreasing reactive oxygen species and destabilizing HIF1α [1]. We are currently examining other metabolic pathways important to cancer that may be regulated by SIRT3.

Conclusions: In conclusion, we show that loss of SIRT3 results in increased glycolysis, which is the metabolic reprogramming observed in some cancer cells and is also known as the Warburg effect.

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P19

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P20

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P21

Loss of *Drosophila* Mondo-Mlx reveals a model for exploring the genetic basis of dietary sugar tolerance

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Background: Sugars are important nutrients for many animals including human, but excessive added sugar might contribute to metabolic diseases. However, we understand little about the genetic factors defining the healthy spectrum of sugar consumption. To secure energy homeostasis in fluctuating nutritional conditions animals possess nutrient sensing mechanisms. In mammals, elevated intracellular glucose flux activates paralogous transcription factors ChREBP/MondoB and MondoA, which together with their binding partner Mlx regulate the expression of target genes involved in lipogenesis and glycolysis [1]. However, the physiological roles and functional conservation of these transcription factors are insufficiently understood.

Materials and methods: To study the *in vivo* function of the Mondo-Mlx system, we have generated a null allele of *Drosophila* *mlx*. To analyze the diet-dependent phenotypes, high protein/low sugar and high protein/high sugar diets were used. Metabolic status of the mutants was explored using metabolomics together with colorimetric metabolite assays. Microarray was done to analyze the gene expression pattern from *in vivo* samples and further confirmed by qPCR. For functional analysis of Mlx target genes RNAi was utilized.

Results: We show that *Drosophila* *mlx* mutant larvae are highly intolerant to dietary sucrose, glucose and fructose, resulting in severe developmental delay and premature death on diets containing moderate to high levels of sugar. The sugar intolerance was phenocopied by RNAi against Mondo. Mondo also physically interacts with Mlx, implying conserved heterodimer function. Metabolic analyses show widespread changes in lipid and amino acid metabolism as well as glucose utilization. Gene expression profiling show changes in metabolic genes, including functional clusters of fatty acid and nitrogen metabolism. Using RNAi we have systematically explored the Mlx-regulated genes for sugar intolerance.

Conclusions: We demonstrate that Mondo-Mlx function is critical in regulating glucose metabolism in *Drosophila*. The heterodimeric function and several metabolic target genes are conserved between *Drosophila* and mammals. This study introduces *Drosophila* as a model for systematic analysis of the genetic basis of sugar tolerance in a physiological setting.

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P22

Is bariatric surgery a solution for obesity?

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Background: The prevalence of obesity in the United States continues to grow at an exponential rate. Obesity is a risk factor for a variety of chronic conditions including diabetes, hypertension, high cholesterol, stroke, heart disease, certain cancers, and arthritis (Flegal et al 2007). The CDC released a report in July 2009 stating the cost of health care related to obesity is as high as \$147 billion annually. In 1998 6.5 percent of medical costs were a result of obesity; in 2009, 9.1 per cent of annual medical costs were a result of obesity-related diagnosis; in 1998 this was 6.5 per cent. The medical costs for an obese person are 42 per cent higher than for a person of normal weight or approximately \$1,429 per year (CDC 2009).

Materials and methods: The purpose of this research is to determine the possible medical benefits of bariatric surgery for patients with a BMI of 35 or greater. The dataset includes 1,217 patients of whom 42 percent are White, 40 percent are Hispanic, 7.2 percent Black, 5.6 percent Pacific Islander, and 2.5 percent Other. The range of age of the patients is 18-75 years. Four different surgical procedures were performed and patients; and the post-operative period ranges from two to 51 months. A substantial portion of patients were morbidly obese (85 percent) prior to surgery, only 15 percent had a BMI below 40. The data gathered included whether the patient was diagnosed and being treated for a specific co-morbidity. The range included cardiac problems, high cholesterol, hypertension, diabetes, incontinence, joint problems, psychological disorders, and respiratory problems. This study focused on those co-morbidities affecting the cardiovascular system, cholesterol, high blood pressure, diabetes and respiratory. The first analysis was completed for individuals that presented with high-cholesterol. The second analysis was completed for individuals that presented with diabetes. The third analysis was for individuals presenting with hypertension. The last analysis was for individuals presenting with respiratory disease co-morbidities. The predictor variables of interest are initial BMI, BMI at 12 months, and type of procedure.

Results: The results of this study show that bariatric surgery reduces BMI; levels of obesity, and reduces the incidence of co-morbidities. The

bypass surgery was most effective with patients achieving weight loss to a normal level. Patients found improvement and/or resolution of pre-existing cardiac issues, high cholesterol, high blood pressure, Type 2 diabetes, and sleep apnea.

Conclusions: Medical costs attributable to obesity are almost entirely a result of costs generated from treating the diseases that obesity promotes, and as long as obesity prevails to the extent that it does today, it will continue to be a significant burden on health care. This research has found improvement in the presence of co-morbidities in post-operative bariatric surgery patients.

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P23

Androgen receptor and nutrient signaling pathways coordinate increased amino acid transport in prostate cancer progression

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Background: Solid tumors including prostate cancer activate angiogenic signals to ensure an adequate blood supply. In parallel, amino acid transporters on the cell surface are also increased so as to provide nutrients for the higher metabolic and growth demands of cancers. We are studying the L-type amino acid transporters (LAT1 and LAT3) that mediate uptake of essential amino acids including leucine. Leucine has recently been shown to be critical for the activity of mTORC1, which regulates protein translation and cell growth. Therefore, increased amino acid transport in prostate cancer cells may drive the mTORC1 signaling pathway to promote unrestrained cellular proliferation.

Materials and methods: We have used the androgen dependent (LNCaP) and androgen independent (PC-3) prostate cancer cell lines to test the role of amino acid transport in cancer. We have used both amino acid uptake inhibitors (BCH) and shRNA knockdown to test the effects on *in vitro* cell growth, cell cycle and signaling pathway analysis as well as *in vivo* bioluminescent tumor growth assays and clinical data correlations with experimental data from primary human cancers.

Results: Our results have demonstrated that prostate cancer cells coordinate the expression of LAT1 and LAT3, thereby increasing leucine uptake to promote mTORC1 signaling and cell growth [1]. We show that inhibition of LAT function leads to decreased cell growth and mTORC1 signaling in prostate cancer cells. These cells maintain amino acid influx via androgen receptor regulation of LAT3 expression, and ATF4 regulation of LAT1 expression after amino acid deprivation. These responses are intact in primary prostate cancer, as indicated by high levels of LAT3 in primary disease, and an increase in LAT1 following hormone ablation and in metastatic lesions. This dynamic regulation of transporter expression is also seen in LNCaP tumor xenograft models, whereby castration decreases LAT3 expression and increases LAT1 expression. Furthermore, shRNA knockdown of either LAT1 or LAT3 significantly decreased tumor growth *in vivo*.

Conclusions: These data show that prostate cancer cells respond to the demand for increased amino acids through an integrated pathway, leading to increased amino acid transporter expression and cell growth. Furthermore, LAT3 and LAT1 may provide novel therapeutic targets in early and late stage prostate cancer respectively.

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P24

Cellular metabolic response to DNA damage

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DNA damage elicits a cellular signaling response that initiates cell cycle arrest and DNA repair. The metabolic response to DNA damage is largely unknown. Here we report a novel metabolic response to genotoxic stress. DNA damage triggers a critical block in the uptake of glutamine, a mitochondrial substrate essential for cellular proliferation. Sirtuins regulate both cellular metabolism and stress responses. We found mitochondrial SIRT4 is involved in the metabolic response to DNA damage. These results suggest that the metabolic adaptation is important for cellular DNA damage response.

P25

Ras-driven cancer cells can scavenge exogenous lipids to support their proliferation

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Background: Lipids are an important structural component of the cell, making up the cell's membranes. Cancer cells need lipids in large quantities to enable their rapid proliferation. Here we aim to quantitatively evaluate the routes by which cancer cells acquire lipids (*de novo* synthesis, uptake from environment) under various conditions (specific oncogenic activations, nutrient availability). We developed a mass spectrometry-based method to quantitate the relative contributions of the various fatty acid acquisition routes and applied this and other methods to study fatty acid metabolism in cell lines, both *in vitro* and *in vivo*.

Materials and methods: Analysis of fatty acid acquisition routes was done by combining ¹³C-tracers with liquid-chromatography mass spectrometry (LC-MS): cells were grown in medium with uniformly labeled ¹³C-glucose and ¹³C-glutamine. Fatty acid samples were generated by saponifying (hydrolyzing) whole cell lipid extracts from these cultured cells, and were analyzed by high resolution LC-MS. Fluxes and relative contributions of fatty acid metabolic events (*de novo* synthesis, uptake, elongation, desaturation) were computed from the observed isotopic patterns. In addition, intact lipid (lipidomics) measurements were done by high resolution LC-MS. These approaches were applied to multiple cell lines with a variety of oncogenic lesions: isogenic transformed baby mouse kidney cell lines (BMK) with either Akt or H-Ras pathway activation, A549 (K-Ras mutant), MDA-MB-468 (PTEN null), and other cancer cell lines. The main findings were corroborated in xenograft experiments.

Results: Determination of fatty acid acquisition routes in an isogenic model with either Akt or Ras pathway activation demonstrated that Akt induces *de novo* fatty acid synthesis, whereas Ras decreases it. This was most evident for the mono-unsaturated fatty acid oleate (C18:1), of which 96% was produced *de novo* in Akt-driven cells but only 57% in Ras-driven cells. We confirmed that Akt pathway activation leads to elevated levels of stearoyl-CoA desaturase 1 (SCD1), a key enzyme in the synthesis of oleate, and that Ras pathway activation leads to increased uptake of exogenous lipids. We hypothesized that SCD1

inhibition would therefore be particularly toxic to the Akt-driven cells. We confirmed that Akt activated cells were significantly more sensitive to SCD1 inhibition than those with Ras activation. Growth of Ras-driven cells was unaffected by SCD1 inhibition for multiple doublings, after which the cells stopped growing. We found that growth inhibition coincided with depletion of lysolipids in the media. This Ras-dependent phenotype was also observed in A549 cells (K-Ras mutant) and other cell lines. A xenograft experiment with A549 cells demonstrated that tumor growth in mice on a diet containing abundant oleate was not affected by SCD1 inhibition, whereas tumor growth was significantly inhibited in mice fed with an oleate poor diet.

Conclusions: This work contributes to explaining at a molecular level how diet may impact tumor progression. In addition, the improved understanding of how cancer cells acquire their lipids will aid future efforts that target lipid metabolism as an anti-cancer strategy.

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Improvement of chemotherapy induced colitis with serum-derived bovine immunoglobulin

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Background: Chemotherapy-induced diarrhea is a common problem, with pain, ulceration, bloating, vomiting and diarrhea occurring in about 40% of patients on a standard regimen and dose [1]. The pathophysiology of this problem is quite complex, involving multiple pathways including increases in pro-inflammatory mediators tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) [2]. Serum-derived bovine immunoglobulin (SBI) has been used for years; first as a valued feed ingredient for weaned pigs where it has been shown to decrease mortality and morbidity and increase growth rates [3]; and then as a safe dietary supplement in humans [4]. In experimental animal models, SBI has been found to decrease both TNF- α and IL-6 in intestinal inflammation, and decrease intestinal permeability [5].

Case history: This patient is a white female, 54 years of age, who developed a renal cell carcinoma and an unrelated adenocarcinoma of the ovary. She underwent a nephrectomy, empiric lymph node dissection, omentectomy, and bilateral salpingo-oophorectomy. A 6-week course of carboplatin and paclitaxel was prescribed for the treatment of the carcinoma of the ovary. Consistent with previous reports, this treatment regimen induced abdominal pain, nausea, vomiting, and diarrhea; resulting in a 12 kilogram weight loss. With the physician's approval, she began using 2 g of SBI b.i.d as a food supplement.

Results: After using SBI, this patient's symptoms improved while she continued with chemotherapy. She gained 2.5 kilograms in the next 2 weeks, without side effects from SBI and was able to successfully complete her course of chemotherapy. SBI has previously been shown to decrease diarrhea and, recently, has been shown to decrease intestinal permeability [6].

Conclusions: SBI may have improved this patient's gastrointestinal complaints and maintenance of body weight. Clinical and nonclinical studies are warranted to determine the efficacy of this food product in decreasing the side effects associated with chemotherapy treatment.

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P27

The cGMP signaling pathway affects feeding behavior in the necromenic nematode *Pristionchus pacificus*

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Background: The genetic tractability and the species-specific association with beetles make the nematode *Pristionchus pacificus* an exciting emerging model organism for comparative studies in development and behavior. *P. pacificus* differs from *Caenorhabditis elegans* (a bacterial feeder) by its buccal teeth and the lack of pharyngeal grinders, but almost nothing is known about which genes coordinate *P. pacificus* feeding behaviors, such as pharyngeal pumping rate, locomotion, and fat storage.

Methodology/principal findings: We analyzed *P. pacificus* pharyngeal pumping rate and locomotion behavior on and off food, as well as on different species of bacteria (*Escherichia coli*, *Bacillus subtilis*, and *Caulobacter crescentus*). We found that the cGMP-dependent protein kinase G (PKG) Ppa-EGL-4 in *P. pacificus* plays an important role in regulating the pumping rate, mouth form dimorphism, the duration of forward locomotion, and the amount of fat stored in intestine. In addition, Ppa-EGL-4 interacts with Ppa-OBI-1, a recently identified protein involved in chemosensation, to influence feeding and locomotion behavior. We also found that *C. crescentus* NA1000 increased pharyngeal pumping as well as fat storage in *P. pacificus*.

Conclusions: The PKG EGL-4 has conserved functions in regulating feeding behavior in both *C. elegans* and *P. pacificus* nematodes. The Ppa-EGL-4 also has been co-opted during evolution to regulate *P. pacificus* mouth form dimorphism that indirectly affect pharyngeal pumping rate. Specifically, the lack of Ppa-EGL-4 function increases pharyngeal pumping, time spent in forward locomotion, and fat storage, in part as a result of higher food intake. Ppa-OBI-1 functions upstream or parallel to Ppa-EGL-4. The beetle-associated omnivorous *P. pacificus* respond differently to changes in food state and food quality compared to the exclusively bacteriovorous *C. elegans*.

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Efficacy of lifestyle interventions in reducing diabetes incidence in patients with impaired glucose tolerance: a systematic review of randomized controlled trials

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Background: Every year over 3.8 million people are dying of diabetes and its complications. Lifestyle intervention was suggested to have beneficial effects in preventing and reducing diabetes incidence. Especially interventions in patients with impaired glucose tolerance (IGT), who belong to a high risk group in developing diabetes, are supposed to be very effective. According to the evidence hierarchy, a 1a level of evidence is missing and therefore a systematic review verifying the efficacy of lifestyle intervention is needed.

Materials and methods: Systematic review: The electronic database PubMed, Embase, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, and Health Technology Assessment database were searched. Main inclusion criteria were randomized controlled trials, impaired glucose tolerance, lifestyle intervention with control group and observation time >6 month. Outcome measures were all diabetes events, as defined by the authors of each study, all-cause mortality, diabetes mortality, and quality

adjusted life years (QALY). Two independent reviewers abstracted the found studies by title, abstract and full-text analysis. Furthermore the reporting quality of each study was assessed by using the CONSORT criteria (Consolidated Standards of Reporting Trials) and the methodological quality by SIGN 50 instrument (Scottish Intercollegiate Guidelines Network methodology checklist for randomized controlled trials). The primary outcome measure was diabetes incidence. Secondary outcome measures were overall mortality, disease-specific mortality, quality adjusted life years (QALY), and clinical parameters; body mass index (BMI), weight change, blood pressure, blood parameter, smoking, alcohol consumption.

Results: 7 trials which included 25 relevant publications were identified [1-25]. Kappa Cohens for title-analysis were $K=0.77$, ($CI=0.71-0.83$), abstract-analysis $K=0.81$ ($CI=0.64-0.92$) and full-text analysis $K=0.78$ ($CI=0.57-0.98$). Overall 5663 patients were analyzed with primary follow-up time: India (3 yr), Japan (4 yr), Sweden (5 yr), Da Qing (6 yr), SIM (3 yr), DPP (5 yr), DPS (4 yr) and drop-out rate ranges from 5% to 28%. Diabetes incidence ranges from 3% to 46% in the intervention group and 9.3% to 67.7% in the control group. The India study reported ARR= 16%, RRR= 29% ($p=0.018$), Japan: ARR= 6.3%, RRR= 65% ($p<0.001$), Sweden: ARR= 4%, RRR= 25% ($p=\text{not significant}$), Da Qing: ARR= 22%, RRR= 32% ($p<0.05$), SLIM: ARR= 20%, RRR= 53% ($p=0.025$), DPP: ARR= 15%, RRR= 58% (significant, no p value reported), and DPS: ARR=12%, RRR= 52% (significant, no p value reported). Mortality and morbidity were only analyzed in Da Qing study which showed no statistical differences (overall mortality: HRR 0.96, CI 0.65-1.41, CVD-mortality: HRR 0.83; CI 0.48-1.40, CVD event: HRR 0.98; CI 0.71-1.37).

Conclusions: Under consideration of heterogeneity in lifestyle interventions and follow up time of the included studies, this systematic review illustrated that lifestyle intervention can have a beneficial effect on the incidence of diabetes in patients with impaired glucose tolerance. However, several studies found the effect of lifestyle intervention decreased after intervention was terminated. Development of standardized lifestyle intervention program is strongly needed and further long term intervention trials using this program are crucial in evidencing the long term efficacy.

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P29

"Tasting" fructose with pancreatic beta-cells: modulation of insulin release by sweet taste receptor signaling and its role in metabolic diseases

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Background: Although glucose is indispensable for the stimulation of insulin release, numerous other insulin secretagogues have been identified. For instance, the dietary monosaccharide fructose potentiates insulin secretion *in vitro*, but the mechanism and physiological significance remains unclear. The T1R2-T1R3 heterodimer of G protein-coupled receptors mediates sweet sensing in the tongue and ablation of either subunit obliterates sweet taste. We hypothesized that the effects of fructose on insulin release may be mediated by sweet taste receptors (TRs) on beta-cells.

Materials and methods: Mice with the homozygous deletion for the T1R2, T1R3, or TRPM5 gene (Dr. Zuker, Columbia University) were bred and genotyped in-house. *In vitro* static insulin release (ELISA, Mercodia) was assessed using cultured human and mouse islets incubated in custom-made wells with various treatments. Fura-2 based calcium imaging was performed using MIN6 cells or dispersed mouse beta-cells. Phospholipase C (PLC) activation was measured using Total Internal Reflection (TIRF) Microscopy in transfected MIN6 cells with a PH_{PLCβ1}-GFP construct. *In vivo* experiments were performed with 8-10 week old catheterized conscious male mice on regular chow after 5-hour fasting. **Results:** Human and mouse islets express sweet TRs. Fructose (10.0mM) rapidly activated PLC and increased intracellular calcium (Ca²⁺) and insulin release at 8.3mM glucose in wild type (WT) islets and MIN6 cells, but these effects were absent in T1R2 knockout (T1R2^{-/-}) islets. Similar to mouse islets, fructose stimulated insulin release in human islets and these effects were blocked by lactisole, a human-specific inhibitor of T1R3. *In vivo*, an intravenous bolus of fructose (1.0g/kg) rapidly increased plasma insulin in WT, but not in T1R2^{-/-} mice. Glucose-stimulated insulin release (GSIS) in WT mice was potentiated by low physiological concentrations of fructose (3.0mM *in vitro*; 0.3g/kg *in vivo*), but these effects were absent in T1R2^{-/-} mice. The transient receptor potential channel M5 (TRPM5) mediates TR signaling in the tongue, contributing to cell membrane depolarization. Islets from TRPM5 knockout mice (TRPM5^{-/-}) failed to increase Ca²⁺ and insulin release in response to fructose. Finally, the expression of TRs is reduced in islets of diabetic mouse models (db/db) and is associated with impaired fructose-induced insulin release.

Conclusions: Fructose is a natural ligand for functional sweet TRs expressed on mouse and human beta-cells. Pancreatic taste receptors sense circulating fructose and activate a distinct signaling pathway involving PLC, TRPM5 and Ca²⁺ influx that potentiates GSIS [1]. Our data together with previous reports showing that sweet TRs in the intestinal epithelium stimulate dietary glucose absorption and GLP-1 secretion, suggest a novel TR-dependent intestinopancreatic axis that participates in the regulation of postprandial insulin release by absorbed sugars. These data suggest a potential link between high-fructose consumption and the development of adverse metabolic effects. Interestingly, beta-cell TR expression and function is reduced in diabetic mouse phenotypes, also suggesting that impaired TR signaling may play a role in the pathogenesis of metabolic diseases.

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P30

SIRT4 controls the balance between lipid synthesis and catabolism by repressing malonyl-CoA decarboxylase

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Lipid metabolism is highly controlled by the nutritional state of the organism. In this study, we identify the mitochondrial sirtuin, SIRT4, as a critical regulator of lipid homeostasis. We find that SIRT4 represses fatty acid oxidation while promoting lipid anabolism. Mechanistically, SIRT4 regulates this balance by inhibiting malonyl-CoA decarboxylase (MCD), an enzyme that produces acetyl-CoA from malonyl-CoA, a precursor for lipogenesis that also inhibits mitochondrial fat oxidation. We find that SIRT4 is active in nutrient-rich conditions, such as in the fed state. As a consequence, SIRT4 null mice display reduced levels of malonyl-CoA in skeletal muscle and white adipose tissue in the fed state and fail to further lower malonyl-CoA levels during fasting. SIRT4 null mice possess a catabolic signature of lipid metabolism and demonstrate decreased *de novo* lipogenesis. These studies highlight SIRT4 as a novel regulator of MCD activity and malonyl-CoA levels, providing new insight into the regulation of lipid homeostasis.

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Screening for metabolic syndrome risk factors in mestizo, tarahumara and menonite scholars from Chihuahua Mexico

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Background: Obesity and diabetes mellitus prevalence has increased during the past decade among adults and adolescents in Mexico. According to la Encuesta Nacional de Salud y Nutrición 2006, 30% of adult population is obese and 39% has overweight, whereas in adolescents is close to 31%. Diabetes prevalence is estimated to be 14.4% in adult Mexican population [1]. Metabolic syndrome (MS) in adults is defined as a cluster of risk factors including abdominal obesity, dyslipidemia, glucose intolerance and hypertension, the presence of three or more components increases the risk for heart disease, diabetes mellitus type 2 and obesity [2,3]. Early identification of children at risk of developing MS must be estimated.

Objective: To screen for MS risk factors among mestizo, tarahumara and menonite teenagers from Chihuahua, Mexico

Materials and methods: A convenient study was performed in high schools from Chihuahua small towns (Guachochi, Cuauhtemoc and Carichi) including 544 teenage students from 12-19 years old, 42% males, 58% females. Blood pressure, anthropometric measures, fasting

glucose, triglycerides and cholesterol HDL were obtained with signed parental informed consent. We used an adapted MS definition [4].

Results: In total population 2.6% had abnormal abdominal obesity, 2.2% had increased fasting glucose, 24.6% had abnormal triglycerides, and 29.4% low HDL cholesterol levels. Within populations, triglyceride abnormal levels were observed in 35% tarahumaras, in 26% mestizos and in 12.9% menonites, being greater in females. HDL cholesterol abnormal levels were observed in 55.4% tarahumaras, in 23.3% mestizos and in 12.3% menonites. Abnormal blood pressure was mainly detected in menonites. According to BMI female percentile classification, tarahumaras showed greater overweight (20.2%), mestizas greater obesity (11.4%) and 15.4% male tarahumaras were overweight. **Conclusions:** This is the first study in Chihuahua that looks for MS risk factors among scholars from different ethnicity. In general, tarahumaras seem to be at higher risk to develop MS, females from this population, had greater overweight, as well as abnormal triglycerides and HDL cholesterol levels. Menonites were healthier than mestizo and tarahumara teenagers. Our results imply that cultural habits and genetic play an important role in developing MS risk factors that we need to consider when prevention strategies are designed.

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P32

Altered metabolic requirements in cancer cell migration and metastasis

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Background: Metastasis poses a great challenge in clinical management of many cancers. Metabolic demands of cancer cell proliferation – i.e. elevated aerobic glycolysis for biomass generation – have been well characterized, but the contribution of altered metabolism to metastasis remains to be elucidated. While elevated aerobic glycolysis, a phenomenon termed Warburg effect, is a hallmark of proliferative tumor cells, emerging evidence suggests that metastatic cancer cells have an opposite phenotype.

Materials and methods: We investigated the metabolism in cell lines of different metastatic capacity. In addition, to quantitatively study cell migration and metabolism, we used MCF10A breast epithelial cells with fluorescent histone tags along with live cell imaging and tracking by a custom MATLAB program to measure speed and behavior of migration. **Results:** Our preliminary data suggest that the more metastatic cancer cells depend on mitochondrial metabolism. Furthermore, changing mitochondrial metabolism in MCF10A breast epithelial cell line affected not only the speed but also the pattern of cell migration. Consistent

with the altered migratory behaviors, stimulation of mitochondrial metabolism changed cell adhesion markers.

Conclusions: In sum, we show evidence that mitochondrial metabolism plays an important role in promoting cell migration and altering cell adhesion with implications for cancer metastasis.

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Glucose uptake via GLUT1 maintains T cell survival during proliferative stress

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Background: Lymphocyte survival is regulated via the balance between pro- and anti-apoptotic BH3 family proteins. *In vitro* this balance is highly dependent on glucose availability. *In vivo*, T-lymphocytes develop in the thymus and then exit to the periphery, where they continually migrate until they encounter cells presenting viral/bacterial antigens. This encounter activates the T cell and induces both proliferation and differentiation into functionally mature T cell subsets. The role of glucose metabolism in regulating cell survival during each of these stages of the T cell life cycle is still unclear; however, we have recently demonstrated that different T cell functional subsets demonstrate distinct metabolic profiles. *In vivo* manipulation of T cell glucose metabolism may therefore represent a novel strategy to manipulate immune responses. In order to explore this area we generated mice with a T cell specific deletion of GLUT1, a major glucose transporter in T cells.

Materials and methods: Mice containing loxP flanked *Slc2a1* (encoding GLUT1) [1] were crossed with mice expressing Cre under a T cell specific *Lck* promoter. Mice with tandem myc tags knocked into an exofacial loop of GLUT1 were generated in-house. Biochemical, metabolic profiling and *in vivo* proliferation assays were performed as described [2].

Results: GLUT1 expression was assayed during T cell development, T cell activation and in mature T cells. Expression of GLUT1 was limited to lymphocytes undergoing rapid proliferation, with only developing and activated T cells exhibiting surface expression of GLUT1. Across the mature T cell subsets, immunosuppressive regulatory T cells (Treg) demonstrated far lower GLUT1 expression in comparison to pro-inflammatory effector T cells (Teff). These differences correlated with differing rates of glucose consumption. Deletion of GLUT1 from developing T cells using a *LckCreSlc2a1*^{fl/fl} mouse model caused a severe reduction in the number of T cells in both the thymus and periphery. One exception to this was the Treg cells, the relative proportion of which increased. Naïve T cells lacking GLUT1 were viable, however when induced to proliferate either *in vitro* or *in vivo* they were unable to correctly upregulate their glucose metabolism, resulting in a misbalance of BH3 family proteins and induction of cell death.

Conclusions: Tight regulation of the glucose transporter GLUT1 is required for normal T cell development and activation. GLUT1 mediated glucose transport is required to drive T cell proliferation and to maintain cell survival. One exception to this is the Tregs, which are far less dependent on GLUT1 for survival. Manipulating GLUT1 mediated glucose metabolism may therefore represent a novel therapeutic strategy to skew T cell responses *in vivo*.

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Plant sterols induce intestinal tumor formation in gender-related manner in ApcMin mice

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Background: Plant sterols are plant derived dietary compounds that are structurally similar to cholesterol. Plant sterols reduce cholesterol absorption, and therefore plant sterol enriched functional foods are designed to lower blood cholesterol level. Reduction of cholesterol absorption increases the level of intraluminal cholesterol, and high intraluminal cholesterol concentration has been associated with enhanced cell proliferation, aberrant crypt formation and tumor formation [1,2]. The aim of this study was to investigate, how plant sterols affect intestinal tumorigenesis, sterol composition of the faeces and the intestinal mucosa, and cell signaling in tumor-prone *ApcMin* mice.

Materials and methods: The *ApcMin* mouse is a well-characterized model for studying associations between dietary factors and colon cancer development. *ApcMin* mice were fed either a high fat, low fiber AIN93-G based control diet (n=12) or a 0.8% (w/w) plant sterol enriched diet (n=12) for 9 weeks. Plant sterol esters were added to the diet in plant sterol enriched margarine. At the end the diet period, intestinal adenomas were counted, and samples were collected for sterol, Western blot, and gene expression analyses.

Results: The number of intestinal tumors increased significantly in plant sterol fed female *ApcMin* mice (46.8±6.7) compared to control female mice (35.0±9.1). In male, there was no difference in the number of tumors between the plant sterol and control group (41.1±8.2 and 36.3±8.5, respectively). No difference in the size of intestinal tumors was observed between the experimental groups. The faecal cholesterol concentration increased by 3.4-fold after plant sterol feeding. The level of mucosal cholesterol decreased in plant sterol fed male compared to control male (-18%, $p=0.01$) and plant sterol fed female (-13%, $p=0.028$). There was no difference in the mucosal cholesterol level in female between groups. Plant sterol feeding increased the level of plant sterols in the intestinal mucosa, and in male resulted in 2-fold higher mucosal sitosterol level compared to female. No difference between groups was found in levels of nuclear cyclin D1 or β -catenin, however, nuclear β -catenin was increased in plant sterol fed female compared to plant sterol fed male (2.2±0.2 and 1.3±0.4, respectively; $p=0.011$). Enrichment of regulated genes belonging to the terpenoid backbone synthesis (KEGG pathway database) was detected after plant sterol feeding in female. The upregulated genes of the terpenoid backbone synthesis (*Mvk*, *Pmvk*, *Idi1*) transcribe enzymes of the mevalonate pathway, which produces cholesterol and isoprenoids for the cell.

Conclusions: Plant sterols accelerated intestinal tumor formation in *ApcMin* mice, and the effect was mainly seen in female. In female, plant sterol feeding upregulated gene expression of several enzymes in cholesterol biosynthesis. Our study suggests that in tumor initiation plant sterol enriched diet has no effect in male but is harmful in female mice.

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P35

Glucocorticoid signaling in the liver and adipose tissue of male and female fructose-fed rats

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Background: The rise in consumption of refined sugars high in fructose appears to be an important factor contributing to epidemic of obesity and metabolic syndrome [1]. Fructose is involved in the genesis and progression of the syndrome through deregulation of metabolic pathways in the liver and adipose tissue, as sites of insulin-modulated metabolism [2]. Enhanced regeneration of glucocorticoids within the liver and adipose tissue, mediated by the enzyme 11 β -hydroxysteroid dehydrogenase type 1 (11 β HSD1), may contribute to adiposity and metabolic disease [3]. 11 β HSD1 reductase activity is crucially dependent on NADPH, a cofactor generated by the enzyme hexose-6-phosphate dehydrogenase (H6PDH) [4]. We hypothesized that harmful effects of high fructose consumption are mediated by alterations in prereceptor metabolism of glucocorticoids and in the level of glucocorticoid receptor (GR) expression and compartmental redistribution in the liver and adipose tissue. We also assume that high fructose intake differently affects glucocorticoid signaling in the liver and adipose tissue of male and female rats.

Materials and methods: Symptoms of metabolic syndrome in 12-week old male and female Wistar rats were analyzed after 9-week intake of 10% fructose solution instead of water. Protein and mRNA levels of 11 β HSD1, H6PDH and GR were determined in the liver and adipose tissue by Western blot and qPCR analyses, respectively. Plasma and tissue corticosterone levels were measured by ELISA.

Results: In male rats, insulin sensitivity was impaired, while blood pressure, non-esterified fatty acid (NEFA) release and plasma triglycerides level were increased by fructose diet. In the adipose tissue, the fructose-provoked increase in corticosterone level was accompanied by enhanced 11 β HSD1 and H6PDH expression, as well as by stimulated GR translocation to the nucleus. In the liver, high fructose diet led to elevation of 11 β HSD1 protein and GR nuclear accumulation, while H6PDH mRNA and corticosterone level were not changed. In fructose fed females, mass of visceral adipose tissue and plasma triglycerides level were increased, while blood pressure and insulin level and sensitivity were unaffected by fructose intake. The rise of corticosterone in the adipose tissue was accompanied by GR protein decline in both the cytoplasm and nuclei.

Conclusions: The results demonstrate that fructose-related elevation of triglycerides and NEFA in blood plasma of male rats coincides with enhanced prereceptor glucocorticoid metabolism in the adipose tissue. The observed gender-specific differences in metabolic phenotype might derive from differences in GR expression and intracellular redistribution in the adipose tissue.

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P36

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in the short-medium term [1,2], although the underlying mechanisms of its efficacy are still not well understood. Despite being a diet in widespread use there are few data available regarding effects on respiratory ratio (RR) [3,4] and resting energy expenditure (REE) and, more specifically, there are no reports about the effects on RR following a return to a non ketogenic diet. The aim of this study was to compare the effects of a 20 day ketogenic Mediterranean diet with phytoextracts (KEMEPHY) and a low-calorie Mediterranean diet (MD) on RR and REE during and 20 days after finishing the ketogenic phase.

Materials and methods: Forty healthy, overweight subjects were recruited and randomly divided into two groups: MD (age 46.61 ± 14.6 , BMI 26.8 ± 2.6 , weight 76.3 ± 9.9 kg) and KEMEPHY (age 50.63 ± 11.6 , BMI 28.8 ± 2.8 , weight 81.8 ± 11.6 kg). KEMEPHY group followed a ketogenic diet (<30 g/day of carbohydrates) using meals that mimic the aspect and the taste of carbohydrates but with virtually zero CHO and with phytoextracts (Tisanoreica®, Lonigo, Italy); after 20 days of strictly KD subjects followed a low carbohydrate non ketogenic diet for 20 days. The MD group followed a standard low-calorie Mediterranean diet (tot Kcal). REE and RR, together with body weight and body composition, were measured in the morning after overnight fasting at the start of the study and after 20 (t_{20}) and 40 days (t_{40}). An Anova test for repeated measures and unpaired t-test with Welch's correction were performed when appropriate.

Results: Compared to starting values RR was significantly decreased in the KEMEPHY group after 20 days ($p < 0.05$) and after 40 days ($p = 0.0002$) (0.86 ± 0.06 ; 0.79 ± 0.05 ; 0.76 ± 0.08 ; respectively) whilst no significant differences were detected in RR in the MED group. No significant differences in REE were detected. Both groups showed a significant decrease in body weight at t_{20} and t_{40} compared to basal conditions (KEMEPHY basal 81.8 ± 11.6 ; t_{20} 77.8 ± 11.4 ; t_{40} 75 ± 11.2 . MED basal 76.3 ± 9.9 ; t_{20} 75.6 ± 9.9 ; t_{40} 71.7 ± 9.8) with the percentage changes in body weight being significantly greater for the KEMEPHY group. Both groups showed a significant decrease in body fat mass at t_{20} and t_{40} compared to basal conditions with the percentage changes in body fat mass being significantly greater for the KEMEPHY group ($P = 0.0135$).

Conclusions: These preliminary data showed that whilst both diet protocols lead to a significant decrease in body weight, the reduction was significantly greater during KEMEPHY. The KEMEPHY diet also lead to a lowering of RR and increased fat oxidation at rest without any effect on REE. These findings suggest that one of the main weight loss mechanisms of KD might be attributed to an improvement in resting nutrient oxidation and interestingly this effect was long lasting, at least for up to 20 days following cessation of the ketogenic. Data on metabolic effects of KEMEPHY 3 months the ketogenic period will soon be available.

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Medium term effects of a ketogenic diet and a Mediterranean diet on resting energy expenditure and respiratory ratio

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Background: Very low carbohydrate ketogenic type diets (VLCKD) have been shown to be more effective for body weight reduction and fat loss compared to balanced or low-calorie Mediterranean diets, at least

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Ursolic acid, a dietary phytochemical, decreases KRAS signaling and modulates cell death pathways in resistant CRC cells

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KRAS mutations are frequent in colorectal cancer (CRC) and have the potential to activate proliferation and inhibit cell death through effects

on MAPK/ERK and PI3K/Akt signaling pathways. Because diet is one of the most important determinants of CRC incidence and progression, we studied the effects of the dietary triterpenoid ursolic acid (UA) on proliferation and cell death induction in human CRC derived KRAS mutated cell lines.

Our results show that UA decreases cell proliferation and induces cell death while decreasing signaling through KRAS as indicated by a decrease in ERK and Akt phosphorylation (western blot). UA also induced cell death.

TP53 mutated cells are known to be resistant to the chemotherapeutic drug 5-FU. Caspase independent apoptosis (Tunel assay), was increased 6 fold by co-incubation of UA with 5-FU. However, apoptosis was only a small percentage of the total cell death induced by UA. In order to explain these observations, we looked into effects on autophagy. Autophagy is emerging as a promising therapeutic target for drug resistant tumors. UA modulated autophagy by inducing the accumulation of LC3 II and p62 levels an effect dependent on JNK activation.

In conclusion, this study shows UA's anticancer potential as a modulator of KRAS signaling and cell death mechanisms increasing sensitivity to the chemotherapeutic drug 5-FU.

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Importance of maintaining redox potential balance in the development of type 2 diabetes

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Background: Accumulation of ROS leads to oxidative stress, which is a common denominator in many diseases. As a result of promising results obtained from *in-vitro* and *in-vivo* studies showing the beneficial function of antioxidants in the prevention of diseases, including benefits of antioxidants on insulin sensitivity and β -cell viability and function, supplementation of antioxidants became very popular. Unfortunately, major randomized clinical trials have yielded disappointing results. Meta analyses conclude that antioxidant supplementations have no beneficial effects on the prevalence of type 2 diabetes (T2D). There are several possible explanations for the failure of antioxidant supplementation to improve health outcomes. Our hypothesis is that there is an optimal redox state that should normally be maintained in cells. If shifted either to the oxidized or to the reduced state, disturbances in β -cell function and in insulin sensitivity of target tissues appear. The aim of this study is to clarify the correlation between redox potential and the development of diabetes.

Materials and methods: Both *in-vitro* and *in-vivo* experiments were conducted. *In-vitro* study was performed on insulinoma RinM and Min6 cell lines. The effects of H₂O₂ and the antioxidant N-acetyl-L-cysteine (NAC) at different concentration were investigated on insulin secretion, viability of β -cells and mRNA expression of specific β -cell transcription factors. *In-vivo* experiments were conducted on KK-A^y mice, a known model of T2D. Mice were given NAC at different concentrations (200-1800 mg/kg/day) during adulthood. Diabetes was evaluated in treated mice by glucose and insulin tolerance tests, histological studies of pancreatic β -cells; insulin signaling pathway was followed in muscle tissue, and several analyses were determined in plasma (insulin, TBARS, TG, cholesterol).

Results: *In-vitro* experiments show that, whereas high concentrations of H₂O₂ (>10 μ M) induce oxidative stress and pancreatic β -cell death, low concentrations (4 μ M) increased viability of these cells, and basal and glucose-induced insulin secretion. High concentrations of NAC reduced viability of cells [1]. mRNA expression of Pdx1 and Pax4 is regulated by the redox state of cells. *In-vivo* results show that while 600, 1200 and 1800 mg/kg/day NAC were all found to improve glucose tolerance of mice, the 1200 mg/kg/day treatment was the most effective in improving insulin sensitivity as indicated by low HOMA-IR. This study clarifies the dose-response effect of NAC on the development of diabetes, and on other manifestations of the metabolic syndrome.

Conclusions: We conclude that alterations in redox balance, resulting from oxidative stress as well as oversupply of antioxidants, may lead to disturbances in the function of pancreatic β -cells and of insulin target tissues.

The study clarifies the beneficial effects of NAC on insulin sensitivity and β -cell function, and suggests that excessive antioxidant consumption has deleterious effects on the development of diabetes. This may provide an explanation for the failure of intervention studies to achieve beneficial health outcomes, and may lead to personalized-adjusted consumption of antioxidants.

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Bioinformatic and molecular investigation of Sirt3 expression

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Background: Fasting and calorie restriction cause significant metabolic changes as organisms try to maintain energy homeostasis. The mitochondrial NAD⁺-dependent protein deacetylase Sirt3 has important metabolic effects, including promotion of fatty acid oxidation during fasting [1] and repression of glycolysis in cancer cells [2]. We sought to investigate the mechanisms by which Sirt3 is transcriptionally induced and regulated using both bioinformatic and molecular methods.

Materials and methods: Our approach was two-pronged: using the DNA sequence analysis program PhylCRM [3], we analyzed the regulatory sequences of Sirt3 and genes with similar expression profiles to determine over-represented transcription factor binding sequences. We also conducted a quantitative real-time PCR-based targeted screen in HEK 293T cells to determine the effects of calorie restriction mimetic drugs on Sirt3 expression levels.

Results: We have identified candidate transcription factors that may affect Sirt3 expression levels, including the zinc finger transcription factor MZF1. We have also analyzed the effect of several drugs on Sirt3 expression, notably observing a decrease in Sirt3 expression with resveratrol treatment.

Conclusions: We have identified transcription factors and calorie restriction mimetic drugs which may control Sirt3 expression and are currently conducting follow-up studies.

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Role of genetic modifiers in Lafora progressive myoclonus epilepsy - a neurodegenerative disorder with defects in carbohydrate metabolism

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Lafora progressive myoclonus epilepsy, also known as Lafora disease (LD), is the most severe and fatal form of progressive myoclonus epilepsy with its typical onset during the late childhood or early adolescence. LD is characterised by the presence of abnormal glycogen inclusions - called the polyglucosan bodies - in the neurons and various other affected tissues. Therefore defects in the glycogen metabolism have been suspected to underlie neuropathology in LD. The two genes known for LD have been extensively characterized; these are the *EPH2A* and *NHLRC1* genes, coding for a protein phosphatase (named laforin) and an E3 ubiquitin ligase (named malin) respectively. Laforin and malin interact with each other and as a complex participate in diverse cellular pathways. The pathways include glycogen metabolism, endoplasmic reticulum (ER) stress, ubiquitin-proteasome pathways and in heat shock response. A number of disease causing mutations, more than 100 of them, are known in *EPH2A* and *NHLRC1* and several studies have looked at possible genotype-phenotype correlations in the LD families. The factors other than the defective LD gene could possibly be involved in modifying the disease onset or the progression in some cases. Indeed, a recent study demonstrates that a sequence variant in the *PPP1R3C* gene coding for the protein targeting to glycogen (PTG) contributes to the milder course of LD. It would be therefore important to check possible contributions of sequence variations in critical regulators of these pathways which interact with laforin and/or malin and test their possible role as modifiers in LD. Here we have extensively reviewed the literature and list potential modifier genes for LD, their cellular functions, and propose their possible effect in LD. Validating these hypothesis will help us in identifying "druggable targets" for delaying the onset and/or the severity of LD if not its treatment.

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Nephrolithiasis and nutrition in obesity

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Background: Obesity is a risk factor for nephrolithiasis (NL). According to an American study of a large cohort with a BMI >30, males increased the risk of NL of 30% and females of 200%. Moreover, diet plays an important role as NL risk factor, mainly in those western countries characterized by a large meat consume. This epidemiology study investigated the NL frequency in an obese Italian population, also considering the relation with metabolic syndrome and interaction with diet.

Materials and methods: Nutritional assessment included: nutritional questionnaires, anthropometry (BMI and waist circumference, body composition estimated by impedance and biochemistry (fasting glucose, serum lipids and transaminases). The presence of

nephrolithiasis, osteoporosis, arterial hypertension, diabetes mellitus and metabolic syndrome were noted. Statistical analysis was performed by the SPSS software.

Results: We studied 532 obese Caucasian subjects (M/F 144/388; age 46±13.1; BMI 29.7±5.85 kg/m²). The stone formers (SF) were 41 (7.7% of the analyzed population, M 9.7%, F 6.9%; age 54.2±11.7 yrs; BMI 30.6±5.8 kg/m²). Non-stone formers (NSF) were 491 (92.3%, M/F 130/361; age 45.3±12.9 yrs [p=0.001]; BMI 29.6±5.8 kg/m²). The percentage of subjects with osteoporosis was higher in SF than in the NSF (15.79% vs 5.84%, p=0.018). In comparison with NSF, SF had higher mean waist size (101.5 vs 96.6 cm, p=0.02), systolic pressure (SF vs NSF, 130.5±15.3 vs 124.4±13.5 mmHg, p=0.007) and higher fasting serum glucose concentration (104±27.4 vs 94±15.3 mg/dL, p=0.02). Diet analysis did not show differences between SF and NSF, except for a higher intake of butter, wine and white meat in SF.

Conclusions: Obese SF had an increased risk of osteoporosis, hypertension, diabetes and metabolic syndrome than obese NSF. This cohort of obese subjects presented a slight increase of prevalence in NL than in general Italian population that is around 5%. The prevalence of stone formers in this Italian obese population is lower than that observed in American obese subjects. This difference could be due to dissimilar genetic background or dietary habits. These findings suggest that Mediterranean diet may have a protective role against NL.

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Nutritional case-control study of Calcium Nephrolithiasis

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Background: It is well established that nutritional habits are relevant in the prevention of Idiopathic Calcium Nephrolithiasis (ICN) and the Mediterranean diet is believed to be protective against nephrolithiasis and not only against cardiovascular events. A case-control study was performed to establish the nutritional habits of Italian ICN patients and the nutritional determinants of lithogenic risk in the considered population.

Materials and methods: Calcium stone formers (SF: n=232, 145 F and 87 M, age 42.25±10.70 yrs, BMI 23.95±3.98 kg/m²) and controls (CTR: n=259, 220 F and 39 M, age 40.97±10.73 yrs, BMI 23.38±3.73 kg/m²) were enrolled. A 3-day nutritional diary was analyzed by the software Dietosystem (DS Medica, Milano, Italy). The nutritional intake was also compared to the Italian nutritional guidelines. Urinary factors were analyzed from 24h urine collection and statistical analysis was performed by the SPSS software.

Results: Urinary data showed an increased excretion of Ca²⁺ (5.55±2.70mg/24h vs 4.12±1.98mg/24h, p≤0.05) and a decreased excretion of K⁺ (49.36±17.81mmol/24h vs 54.55±17.2mmol/24h, p≤0.05) and citrate (544.29±262.82mg/24h vs 660.09±247.21mg/24h, p≤0.05) in SF than CTR. Nutritional analysis found differences between SF and CTR: a higher caloric intake (2013.03±753.77 Kcal/die vs 1933.39±502.20 Kcal/die, p≤0.05), a higher total protein intake (79.8±22.70 g/die vs 75.6±21.31 g/die, p≤0.05) and a higher vegetable protein intake (25.74±9.41 g/die vs 22.94±8.73 g/die, p≤0.05) were observed in SF than CTR. Moreover SF showed a higher intake of sodium, oxalate, complex carbohydrates, purines, arachidonic acid and a higher acid load. The comparison of SF nutritional intake to the Italian guidelines demonstrated a prevalence of hyperproteic, hyperlipidic and hypercaloric diet, a low calcium, potassium and fiber intake, and a high salt, phosphorus and oxalate intake. Water intake covered the recommended quantity (1.5-2 L/die) for healthy population, but not for SF (>2 L/die).

Conclusions: In conclusion, our study found different nutritional habits between SF and CTR and confirmed some dietetic errors respect to national dietetic recommendations. These errors could increase the risk to develop ICN in subjects with a lithogenic genetic background and confirm the usefulness to give nutritional advices to SF patients.

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Catechin-rich green tea extract increases serum cholesterol levels in normal diet- and high fat diet-fed rats

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Background: *In vivo* studies using rodents have shown that green tea extract and catechins isolated from green tea can induce a variety of health effects, including anti-obesity, hypoglycemic and hypolipidemic activities [1]. These beneficial effects of green tea have been observed in experiments using high fat, high cholesterol and high fructose diet-fed animals. In the present study, we examined the effects of catechin-rich green tea extract on serum glucose and lipid levels in normal diet- and high fat diet-fed rats.

Materials and methods: Catechin-rich (30% catechin) green tea extract (GT) was used for *in vivo* studies. Male Sprague-Dawley rats (average weight 232.9 g) were divided into six groups containing six rats each. The first group was fed on a normal diet (ND, 10% calories from fat); the second group on a high fat diet (HFD, 40% calories from fat); third group with ND containing 1% GT (ND + 1% GT); fourth group with HFD + 1% GT; fifth group with ND + 3% GT; and the sixth group with HFD + 3% GT. After four weeks of feeding, rats were euthanized by whole blood collection under anesthesia. Total RNA samples extracted from the liver were used for microarray analysis.

Results: Body weight was significantly lower in GT-containing diet-fed rats than that in GT-free diet-fed rats regardless of whether they received ND or HFD (Table 1). As expected, GT reduced serum glucose (Glc) and triglycerides (TG) levels in ND and HFD-fed rats but was GT concentration dependent (Table 1). Diets containing 1% GT did not affect the serum levels of total cholesterol (T-Cho) and high-density lipoprotein cholesterol (HDL-Cho) significantly, although there was a trend towards an increase in cholesterol levels. When 3% GT was added to the diet, the serum levels of T-Cho and HDL-Cho increased significantly in ND and HFD-fed rats compared to non-GT fed rats (Table 1). The degree of increase in the levels of these serum factors was higher in ND-fed rats compared with HFD-fed rats. Serum AST and ALT levels suggested that hepatic damage induced by GT feeding had not occurred (Table 1). Preliminary microarray analysis data suggested that mRNA levels of more than half of the genes involved in cholesterol synthesis were increased and the mRNA levels of *Cyp7a1*, which is involved in bile acid synthesis, was decreased in ND + 3% GT-fed rats compared with GT-free ND-fed rats.

Conclusions: The results from the current study suggest that GT can increase serum cholesterol levels, especially in ND-fed rats, when it is consumed in excess. This effect may partly occur through changes in liver gene expression induced by GT feeding. Further studies are required to evaluate whether the effects of GT are beneficial or harmful to health.

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Blood levels of the micronutrient zinc decrease with advancing age in normally nourished elderly persons

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Background: The prevalence of malnutrition in elderly populations has emerged as a social issue. Malnutrition can cause significant deterioration of health, impede individuals' independence and quality of life, and increase morbidity and mortality rates. Early detection and prompt treatment are very important in preventing malnutrition. The Mini Nutritional Assessment (MNA[®]) is now widely used for nutrition screening and as an assessment tool for elderly persons aged over 65 years. In this study, we investigated which blood nutrients were liable to decrease with advancing age in healthy elderly persons who were classified as normally nourished.

Materials and methods: The participants comprised 13 elderly adults (7 men and 6 women, mean age 82.5±5.2 years), belonging to a senior citizens' club in Ichikawa City, Japan. An individual's physical composition was analyzed using anthropometric parameters. The Nutrition status Mini Nutritional Assessment comprising 18 questions (the full MNA[®]) and a brief-type self-administered diet history questionnaire (BDHQ) were used to evaluate the nutritional status and dietary intake of subjects, respectively. The levels of various nutrients in the blood were determined by laboratory biochemical blood tests. Measurements were performed in December 2011 and the data were compared with data obtained from the same participants two and half years ago (March 2009) [1].

Results: The average screening score of the full MNA[®] from March 2009 and December 2011 was 26.7 ± 1.3 and 27.5 ± 1.3, respectively. The estimated nutrient intake measured by BDHQ analysis revealed all participants were well nourished. The Body Mass Index score from December 2011 (23.0 ± 1.8) was slightly but significantly lower (*p* < 0.01) than that from March 2009 (23.5 ± 2.0). Although the concentration of red blood cells, hematocrit, hemoglobin content, and serum levels of total protein, albumin, and iron were not significantly different compared with the original measurements (two years and nine months previously), these values appeared to be declining. However, the levels of serum zinc had decreased by 22% during the last 2 years 9 months (*p* < 0.001), although levels were still within the normal range.

Conclusions: The results presented here suggest that elderly people might become susceptible to zinc insufficiency with increasing age, even if they are considered well-nourished. Zinc deficiency causes significant impairment of both the adaptive and innate immune responses, and is important for promoting systemic inflammation [2]. Therefore, it is important for nutritionists to monitor the consumption of micronutrients such as zinc more carefully, in order to maintain and enhance the quality of life in elderly people.

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Table 1 (abstract P47). Impact of green tea extract on body weight, liver weight, serum glucose, serum lipids, serum AST, and serum ALT in rats fed an ND or a HFD diet¹

Variables	ND	HFD	ND + 1% GT	HFD + 1% GT	ND + 3% GT	HFD + 3% GT
Body weight (g)	370 ± 17.4	417 ± 11.1 [*]	347 ± 9.8 ^{*,‡}	399 ± 12.6 ^{*,‡}	309 ± 9.1 ^{*,‡}	341 ± 18.1 ^{*,‡}
Liver weight (g)	11.1 ± 1.0	12.9 ± 1.8	9.4 ± 1.1 ^{*,‡}	11.1 ± 1.1	8.2 ± 0.3 ^{*,‡}	9.0 ± 0.6 ^{*,‡}
Glc (mg/dL)	200 ± 29.6	184 ± 29.5	167 ± 40.2	156 ± 37.4 [*]	131 ± 22.5 ^{*,‡}	140 ± 22.9 ^{*,‡}
TG (mg/dL)	62.6 ± 24.9	76.3 ± 12.2	39.8 ± 5.9 [‡]	52.3 ± 11.6 [‡]	30.2 ± 21.6 ^{*,‡}	38.5 ± 7.2 ^{*,‡}
T-Cho (mg/dL)	57.1 ± 6.1	55.2 ± 5.8	61.0 ± 8.5	65.2 ± 6.9	84.6 ± 7.3 ^{*,‡}	70.3 ± 9.4 ^{*,‡}
HDL-Cho (mg/dL)	36.3 ± 6.0	33.4 ± 5.9	38.3 ± 5.6	38.0 ± 3.9	58.8 ± 11.0 ^{*,‡}	46.7 ± 7.6 ^{*,‡}
AST (U/L)	94.3 ± 32.1	87.5 ± 30.5	88.7 ± 23.8	71.8 ± 25.6	78.7 ± 11.4	69.0 ± 15.6
ALT (U/L)	12.0 ± 2.5	14.0 ± 5.5	13.2 ± 2.1	10.5 ± 5.2	17.5 ± 3.1 [*]	15.0 ± 3.8

¹Data are means ± SD, n=6. **p* < 0.05 versus ND. [‡]*p* < 0.05 versus HFD.

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Metabolic defects induced by high-fat feeding in mice are rapidly reversed by a low-fat diet

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Background: It is well established that high-fat feeding increases adiposity and impairs glucose metabolism in mice. The aim of this study was to determine the extent to which these changes are reversible if the high-fat diet (HFD) is removed.

Materials and methods: C57Bl6 mice were fed a low-fat chow diet (LFD) or a HFD (45% calories as fat) for 9 weeks and body composition, glucose tolerance and tissue triglycerides were assessed. A group of fat-fed animals were then switched to a LFD (HFD-LFD) and after 4-5 days their body composition and glucose tolerance were reassessed.

Results: Mice fed the HFD displayed a 73% increase ($P < 0.01$) in whole-body fat mass, an 80% elevation ($P < 0.01$) in muscle and liver triglyceride levels and a substantial impairment in glucose tolerance compared to animals fed the LFD (area under curve during GTT: 1166 ± 76 vs. 506 ± 47 mM.min, $P < 0.001$). The switch to a LFD resulted in a transient decrease in total caloric intake, but only a small drop in body weight (31.3 ± 0.8 vs. 30.3 ± 0.5 g, pre vs. post, $P = 0.06$). Despite the minimal change in body weight, whole-body fat mass in the HFD-LFD group was reduced almost to the level of LFD controls (12.7% vs. 14.2% by DXA, LFD vs. HFD-LFD). Consistent with the reduction in whole-body adiposity, glucose tolerance (AUC during GTT: 510 ± 49 mM.min) and muscle and liver triglycerides in the HFD-LFD animals were also restored to the level of LFD animals. Intriguingly, a separate group of mice that were pair-fed HFD to match the drop in caloric intake in the HFD-LFD group, displayed no improvements in glucose tolerance or tissue triglyceride levels.

Conclusions: Our findings suggest that the metabolic defects induced by high-fat feeding in mice are rapidly reversible if animals are switched to a LFD.

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Appliance of domiciliary nutrition instruction method for the elderly people requiring long-term care in Japan

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Background: In Japan, elderly people aged 65 and over account for more than one fifth of the nation's population. To add to this, the number of aged households is increasing year by year. Because elderly people are preferential to have tender and non-fatty foods in general, they are prone to biased nutrition status. According to the result of a National Health and Nutrition Survey, the low weight male who aged 60-69 and over percentage has increased from 7.2% (1987) to 12.3% in 2009. Also, female is 18.6% to 22.3%. Therefore both male and female has increased about 5%. It is predicted that nutritional management for elderly person requiring long-term care but living in their home may become difficult. Establishing the novel nutritional guidance for such elderly person with their favor is one of the most important challenges for clinical dietitian in Japan. In this study, we carried out two questionnaire surveys, in order to understand nutritional status of elderly people requiring long-term care but living in their home and find out dietary preference of elderly people.

Materials and methods: In order to grasp the present condition of care of elderly people requiring long-term care, we performed the questionnaire by mail to 197 nursing-care-services entrepreneurs in the part of Tokyo. In order to be and to grasp the present condition

of house elderly people's meal, both the part of Tokyo and one of city in the Chiba used semi-quantitative food frequency questionnaire (FFQ) method which set the elderly people aged 65 and over as the 147-person object. Also, we performed the questionnaire about the cooking method also went simultaneously.

Results: According to the nursing-care-services entrepreneur's questionnaire, there was a problem as decrease of dietary intake which is 78.0%, decrease of weight of 39.6%, malnutrition of 54.9%. According to FFQ, it was characteristic that people intake of fish and shellfishes more than meat, also soft food, such as a sweet roll. Moreover, in investigation of the cooking method, there was much ingestion of the sliced raw fish, and it was simple cooking method, people would rather cook as bake.

Conclusions: When the decrease of dietary intake is seen in connection with aging, it is necessary to recommend food with high nutrient density. Moreover, it is also important to propose the meal content which took dysphagia function into consideration enough. It is important that dietitian recommends simple recipes using microwave oven to elderly people and their family, rather than telling recipes for elaborate meals.

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The increase of NADH fluorescence lifetime is associated with the metabolic change during osteogenic differentiation of human mesenchymal stem cells (hMSCs)

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Background: Fluorescence lifetime of NADH had been proposed to use as an intrinsic biomarker for monitoring cellular metabolism. In our pervious studies, we have demonstrated that NADH lifetime of hMSCs increase gradually with time of osteogenic differentiation. In this study, we performed NADH lifetime measurement of hMSCs from a different donor and studied the association with several metabolic indices such as ATP level, oxygen consumption and lactate release. We also measured the quantity of Complex I, III, IV and V during hMSC differentiation.

Materials and methods: NADH fluorescence lifetime images were performed as our previous studies [1]. In brief, treated hMSC cells were imaged with a two-photon laser scanning microscope and with a 60×1.45 NA PlanApochromat oil objective lens (Olympus Corp., Japan). NADH fluorescence was excited at 740 nm by a Verdi pumped modelocked femtosecond Ti:sapphire laser (Coherent, Inc., Santa Clara, California) at 76 MHz and the emitted fluorescent light was detected at 450 ± 40 nm by a bandpass filter (Edmund Optics, Inc., Barrington, New Jersey). Fluorescence photons were detected by a photon-counting photomultiplier H7422P-40 (Hamamatsu Photonics K.K., Hamamatsu, Japan). Time-resolved detection was conducted by the single-photoncounting SPC-830 printed circuit board (Becker & Hickl GmbH, Berlin, Germany). Data were analyzed with the commercially available SPCImage v2.8 software (Becker & Hickl GmbH, Berlin, Germany) via a convolution of the two component exponential decay function and the instrument response function (IRF), and then the convolved result were fitted to the actual data to derive lifetime parameters τ_1 (NADH short lifetime component), τ_2 (NADH long lifetime component), a_1 (amplitude related to τ_1), a_2 (amplitude related to τ_2), and τ_m . Mean lifetime τ_m is defined as $(a_1\tau_1 + a_2\tau_2)/(a_1 + a_2)$. IRF was measured using a second-harmonic generated signal from a periodically poled lithium niobate crystal. The cell respiration rate was measured by a 782 Oxygen Meter as previously reported [2] and intercellular ATP level was measured by the bioluminescent somatic cell ATP assay kit (Sigma-Aldrich, St. Louis, Missouri).

Results: The results show that during differentiation more oxygen consumption, higher ATP level expressed and less lactate released. Similar to our previous study, NADH fluorescence lifetime increased gradually during osteogenic differentiation and until 4 weeks after differentiation The increase of NADH lifetime was associated with

ATP level and oxygen consumption ($R^2 = 0.88$ and 0.95 respectively). Significant higher expression of the total Complex protein than controls was observed at 3 and 4 weeks after differentiation. However, Complex I expression, which was believed to directly related to NADH, did not show significant correlation with the increase of NADH fluorescence lifetime. In summary, we demonstrated that the change of NADH lifetime was associated with the metabolic change during osteogenic differentiation of hMSCs. The increase of NADH lifetime was in part due to the increased Complex protein interaction in mitochondria during differentiation.

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P52

Inhibition of myostatin signaling increases glucose in insulin-deficient diabetic mice

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Background: Myostatin (MSTN), a TGF- β superfamily member, is a negative regulator of muscle mass that plays an important role in metabolism. *Mstn* KO mice have increased muscle mass, reduced adipose mass and improved insulin sensitivity. We have recently shown that MSTN inhibition in muscle prevents the development of diabetes in a mouse model of lipodystrophy. Whether inhibition of MSTN in a type I diabetes model would improve hyperglycemia is unknown.

Materials and methods: We used streptozotocin (STZ)-treated C57 mice in which the insulin-producing β -cells were specifically damaged leading to hyperglycemia. After overt diabetes developed, the STZ-treated mice were injected with a MSTN inhibitor, a soluble Activin receptor type II B (ACVR2B:Fc). Blood glucose levels were measured regularly by glucometer. Pyruvate tolerance and glutamine tolerance tests were performed and several hormones in the serum were measured. Real-time PCR was used to compare the expression level of some genes involved in gluconeogenesis.

Results: The soluble ACVR2B:Fc-treated STZ mice have higher blood glucose levels compared with untreated STZ mice. There were no differences in insulin and glucagon levels between ACVR2B:Fc treated or untreated STZ mice. However, there were higher levels of the glucocorticoid corticosterone in soluble ACVR2B:Fc-treated mice. Real-time PCR data showed that the expression of the *PEPCK* gene was increased significantly in ACVR2B:Fc-treated mice.

Conclusion: Our data suggest that the soluble ACVR2B:Fc treatment worsens hyperglycemia possibly due to increased gluconeogenesis. These data suggest that MSTN inhibition will not be useful for treating type I diabetes.

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Tissue inflammation and NCDs: dietary control, physical exercise and mind body interactions

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Background: Cause and effect of dietary constituents on the genesis of NCD epidemics (CVD, diabetes, etc) in the Western developed and some developing countries has been difficult to unravel. It appears that

inflammation is a key factor and that in its absence total cholesterol may have an apparently neutral effect on arterial tissues and the myocardium. The Mediterranean-style diet, combined with physical exercise, resembles a notional Palaeolithic diet of a pre-agricultural era in that the ratio of omega-3/omega-6 unsaturated fatty acids is higher than in most Western diets [1]. Furthermore, evidence suggests that this higher ratio can beneficially change brain function and its interaction with gut, liver and heart and influence mind-body interactions. This group, in collaboration with others, seek to test the hypothesis, albeit for a 4y longitudinal psychosomatic randomised placebo control vs. omega-3 intervention trial of 90 subjects, 30 each in adolescent, adult and elderly groups of men and women in Poland, that this higher ratio can not only favourably change the rhythmic characteristics of blood pressure and heart rate, but beneficially improve mental health. However, a 3m feasibility study has been undertaken to test methods and safety associated with the main protocol the underpinning objective being that a return to the original balanced (1:1) ratio of polyunsaturated fatty acids (PUFAs) and/or to a corresponding 25% proportion of $\omega 6$ highly unsaturated fatty acids (HUFAs) in plasma/serum total lipids ($\omega 6:\omega 3$ PUFAs = 1:1 and/or $\omega 6$ in HUFAs = 25) can possibly reduce the risk of developing chronic degenerative diseases towards zero at the population level.

Materials and methods: Subjects (consented): 5 family-related members + 4 family acquainted subjects were arranged in the three age groups. Clinical (published elsewhere [2]): General medical examination with blood and urine tests; psychological health assessment using Eysenck's Personality Questionnaire (Polish); body mass composition; ambulatory blood pressure monitoring; blood lipids were the main methods.

Results: All subjects reported subjective mind and body health improvements concerning cognition, concentration, communication, breathing, general feeling, relief of dizziness and pain confirmed by medical, biochemical and socio-psychological analyses. In all three subgroups, prolonged w-3 HUFA intake appears to maintain the new status, not to further reduce it. The main results were: 1. Daily intake of w-3 HUFA needed to reverse the Omega-6 Status from 75 to 50% (3.35g w-3 HUFA /day) and then from 50 to 25% (3.35g w-3 HUFA/day) in the blood-tissue of a modern Western population was redefined; 2. Benefits of the presence of a "blunter" (LIPISTASE) of inter-individual genotypic variance were indicated; 3. A reduction in daily intake of omega-6 fatty acids from plant and animal origins is probably needed to augment post-intervention to reach the evolutionary selected blood/tissue ratio; 4. age and gender, body composition, metabolic rate and lifestyle did not substantially affect the linear relationship between the change in w6S and omega-3 intervention dose. Safety appears not to be an issue.

Conclusions: Pilot data supports, with amendments, the implementation of the 4y longitudinal randomized placebo vs. omega-3 intervention trial.

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P54

Metabolic modeling predicts perturbations extending lifespan in yeast and counteracting aging in mammalian muscle

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Background: Disease is classically viewed as a disruption of healthy homeostasis. This naturally gives rise to the quest to find drugs that

can efficiently transform a disease state back to a healthy one. Here we address this challenge on a genome scale for the first time. We chose to focus on aging, aiming to predict perturbations (both genetic and environmental) that can extend the organism's lifespan. Aging forms a nice test bed to examine our approach, since it is typically accompanied by progressive changes in gene expression. Furthermore, Caloric Restriction (CR), a dietary intervention that extends lifespan and delays the onset of age-associated phenotypes, is known to reverse these expression changes [1, 2]. As CR has very limited value as a therapeutic regimen, these findings strongly motivate the search for metabolic drug targets that can reverse the metabolic state of the aging to that of the young.

Materials and methods: Here we present a novel Metabolic Transformation Algorithm (MTA) that given a source (disease, e.g., aged) and a desired target (healthy, e.g., young) metabolic state, identifies the genetic or environmental perturbations that best enable a transformation from the source to the target state. The MTA algorithm works in the realm of metabolism and is based on Constraint-Based Modeling, an increasingly widely used computational method for studying metabolism on a genome-scale [3, 4].

Results: First, the prediction accuracy of MTA has been extensively validated using data from known perturbations in *Escherichia coli*, *Saccharomyces cerevisiae* and mammalian cell lines. Second, Analyzing gene expression data in aging *Saccharomyces cerevisiae*, seven novel lifespan-extending metabolic targets predicted by MTA were further tested experimentally. Two of those were successfully validated (a 10-fold increase over their expected frequency), one of them extending lifespan markedly by about 50%. Analyzing mammalian aging muscle expression data, MTA identifies novel drug targets transforming the metabolic state to that of the young, highlighting the role of a key inflammatory pathway of Eicosanoids metabolism. These predictions are enriched with human orthologs of known lifespan-extending genes in *Saccharomyces cerevisiae* and *Caenorhabditis elegans*.

Conclusions: MTA offers a fundamentally new approach for identifying metabolic drug targets in a broad span of major metabolically-related human disorders, including obesity, neurodegeneration and cancer. As MTA aims to retrieve the metabolic state back to its normal homeostasis, one may expect that it may lead to new drugs with lesser side-effects.

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P55

Effect of nano-encapsulated (-) epigallocatechin gallate on triglyceride accumulation in 3T3-L1 adipocytes

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Background: Obesity is considered the major risk factor for type 2 diabetes mellitus (T2DM), cardio-vascular diseases like hypertension and atherosclerosis [1]. Catechins are the major source of flavonols and they are comprised of various types of catechins such as epigallocatechin 3 gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) [2]. Numbers of *in vivo* studies have reported weight loss after drinking green tea for several months. To support these *in vivo* findings, many *in vitro* studies demonstrated effect of pure EGCG which is an important biologically active green tea catechin in reducing triglyceride accumulation in adipocytes [3]. However, these anti-adipogenic properties of EGCG are greatly dependent on bio-availability which is

very low in animals as well as in humans. One study showed that, peak plasma concentrations in humans were 1.3 µM for EGCG after a single dose of 1.5 mmol through oral administration [4]. Nano-encapsulated EGCG (Nano-EGCG) may help to increase bioavailability and to exert maximum anti-adipogenic effects.

Materials and methods: We synthesized Nano-EGCG using sonication method. Pure EGCG (95%) was bought from SIGMA Company. Nano-EGCG was dissolved in phosphate buffered saline (1XPBS) and was stored at 4 degree Celsius. 3T3-L1 Preadipocytes were obtained from ATCC. Cells were cultured using a standard protocol. Post-confluence cells were differentiated using a standard chemical cocktail mixture. Further cells were maintained in maturation medium and treated with native EGCG and Nano-EGCG at 10 µM for 48 hours. Cellular triglyceride levels were measured using an enzymatic method.

Results: Nano-EGCG significantly ($p < 0.05$) reduced triglyceride accumulation in mature adipocytes as compared to 1X PBS, native EGCG and its void counterpart. EGCG didn't significantly reduce the cellular triglyceride accumulation as compared to 1XPBS.

Conclusion: Data suggests that 10 uM of Nano-EGCG significantly reduced triglyceride accumulation as compared to 1X PBS, void counterpart and native EGCG.

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P56

Modeling effects of diabetes and obesity co-morbidities in endometrial cancer development and progression

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Background: Diabetes and obesity are well established risk factors for the development of endometrial cancer. We hypothesized that circulating cytokines associated with diabetes and obesity exert direct effects on primary human endometrial cell proliferation and transformation and worsen pathology in endometrial cancer patients.

Methods: The effects of insulin and insulin like growth factors 1 and 2 (IGF1 and 2) on proliferation were measured in primary cultures of human endometrial cells using a cytotoxicity assay, while transformation was measured by soft agar colony formation and histologic evaluation of and organotypic model of endometrial carcinogenesis and chemoprevention [1]. Akt phosphorylation status was measured by Western blot. Levels of leptin, adiponectin, adipin, c-peptide, tumor necrosis factor α (TNFα) chemokine (C-C motif) ligand 2 (CCL2, also called monocyte chemoattractant protein-1 or MCP-1), Interleukin (IL)-1β, IL10, vascular endothelial growth factor (VEGF) and insulin in serum from 84 endometrial cancer patients were measured using multiplex magnetic bead assays (BioRad and Millipore). Glucose was measured using a kit (Cayman), and HOMA Scores were calculated. These biomarkers were compared with tumor stage, grade, recurrence of disease and age at diagnosis using Spearman Correlation.

Results: Insulin, IGF1 and IGF2 caused dose-responsive induction of endometrial cell proliferation, which surprisingly was associated with

loss of Akt phosphorylation on Ser473. Insulin treatment as a single agent induced anchorage-independent colony formation in the soft agar assay and histologic features of transformed cells in organotypic cultures. Insulin also enhanced these transformation measures induced by 7,12-Dimethylbenz(a)anthracene (DMBA) carcinogen. Elevated CCL2 in serum of endometrial cancer patients was significantly associated with worse stage of disease ($r=0.262$, $p=0.018$). Reduced serum TNF α was significantly associated with increased age at diagnosis ($r=0.332$, $p=0.048$). No other associations between biomarkers and patient characteristics were noted.

Conclusions/discussion: Insulin, IGF1 and IGF2 have direct effects on normal endometrial cell proliferation and transformation potential implicating these cytokines in the increased risk of endometrial cancer associated with diabetes and obesity. The decreased TNF α found to be associated with increased age at diagnosis may be due to the known inverse association of TNF α with age and insulin levels. The association of elevated CCL2 with tumor stage suggests that direct effects of CCL2 on tumor and surrounding stromal cells over-rides CCL2 recruitment of T-lymphocytes into tumors to enhance tumor-specific immunity. Follow-up studies are planned to validate the biomarker results in an independent set of specimens and to evaluate the role of Akt signaling in insulin and CCL2 effects on endometrial carcinogenesis and progression.

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P57

Sirt1 promotes adipogenesis by binding to and inhibiting Sirt1

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Background: Members of the mammalian sirtuin family, Sirt1 – Sirt7, are known to regulate metabolic processes especially carbohydrate and fat metabolism [1, 2]. Sirt1 and Sirt2 inhibit adipocyte differentiation [3, 4] while Sirt1 and Sirt6 prevent liver steatosis [5]. These examples illustrate a synergistic action of different sirtuins in promoting lean, “healthy” phenotypes. We have previously shown that Sirt7 knockout mice display signs of premature aging, suffer from progressive cardiomyopathy and have a reduced lifespan [6]. Here, we investigate the biological function of Sirt7 in the regulation of metabolism in white adipose tissue (WAT) and liver.

Results: To discover new regulators of Sirt1 activity we performed an unbiased screen for molecules that might interact with Sirt1 using a label free quantitative mass spectrometry based co-immunoprecipitation strategy. We identified Sirt7 as a novel Sirt1 binding protein. The interaction between Sirt1 and Sirt7 was confirmed by immunoprecipitation of endogenous proteins and GST pull-down assays. Sirt1 protein expression and enzymatic activity was increased in WAT of Sirt7 knockout mice leading to age-dependent lipodystrophic phenotype. Increased Sirt1 activity might account for resistance of Sirt7 knockout mice fed high fat diet against liver steatosis. In vitro experiments demonstrated a diminished ability of Sirt7 deficient MEFs and primary preadipocytes to undergo adipogenesis. These defects were rescued by knock-down of Sirt1 or in cells deficient for one Sirt1 allele (Sirt1 $^{+/-}$; Sirt7 $^{-/-}$).

Conclusions: Our results highlight the importance of cross-regulatory circuits among individual members of the sirtuin family in organismal homeostasis. Lack of Sirt7 leads to a sustained activation of Sirt1. Apparently, such un-physiologically exaggerated, persistent Sirt1 activation results in metabolic dysfunction and nullifies its principally beneficial effects such as fat mobilization and inhibition of adipogenesis.

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P58

Aberrant iron homeostasis, oxidative fiber enrichment, and activation of ketogenesis in muscle tissue of ISCU Myopathy patients

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ISCU Myopathy, a disease characterized by life-long exercise intolerance and impaired mitochondrial oxidative metabolism, is caused by deficiency of the Fe-S cluster scaffold protein ISCU. We performed gene expression analysis on muscle biopsies from ISCU Myopathy patients to elucidate which molecular processes were transcriptionally remodeled in response to impaired Fe-S cluster assembly. We found that ISCU depletion led to increased expression of the mitochondrial iron importer MFRN2 and the rate-limiting heme biosynthetic enzyme ALAS1. Gene expression and histologic studies demonstrated that patient muscle composition was shifted towards fewer glycolytic muscle fibers, more oxidative fibers, and increased capillary abundance. Paradoxically, mitochondrial fatty acid uptake and oxidation genes were coordinately up-regulated in patient muscles despite dramatic impairments of aconitase and succinate dehydrogenase activities. The ketogenic enzymes HMGCS2 and BDH1 were also significantly up-regulated, as was the secreted starvation response hormone, FGF-21. We propose that ketogenesis may be initiated to restore free coenzyme A levels and shunt fatty acid oxidation products to distal respiration-competent tissues when TCA cycle and/or respiratory chain function is sufficiently impaired in affected patient muscle fibers. Moreover, our work shows that plasma FGF21 is a sensitive non-invasive biomarker of ISCU Myopathy, in addition to other mitochondrial myopathies.

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Abstract not submitted for online publication

P60

A pilot safety-feasibility dietary trial targeting insulin inhibition in ten patients with advanced cancer

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Background: Hyperinsulinemia, Diabetes type 2, and obesity have been identified as increased risk factors for a variety of cancers [1].

Conversely, insulin inhibition (INSINH) can plausibly limit cancer growth by demonstrated mechanisms including ketosis [2] and regulation of downstream signaling proteins such as mTOR (inhibition) and AMPK (amplification), already in development as drug targets [3,4]. Increased ^{18}F -2-fluoro, 2-deoxyglucose (FDG) uptake on positron emission tomography (PET) scan is characteristic of many aggressive malignancies. We examined safety and feasibility of a four week INSINH diet in patients with advanced PET positive cancers, and compared exit vs. baseline PET scan changes as surrogate measures for tumor response.

Methods: Eligible patients, referred by faculty or self-referred after locating our trial (e.g. www.clinicaltrials.gov/NCT00444054), had failed or refused ≥ 2 standard chemotherapy courses and demonstrated FDG-positive scans on baseline PET. Exclusions included concurrent chemotherapy, end-organ disease, hypoglycemic medications, difficult compliance, or BMI < 20. A supervised INSINH diet restricting starches and sugars for 28 days, was monitored weekly for macronutrient intake, body weight, serum electrolytes, betahydroxybutyrate concentrations [BHB], [insulin], [IGF1,2]. An exit four-week PET was obtained for comparison with the baseline scan.

Results: Ten subjects with diverse cancers completed > 26 days of INSINH without associated unsafe adverse effects. Mean caloric intake decreased (35 ± 6) % vs. predicted requirements despite best efforts to encourage increased food consumption. Weight loss (median 4%, range 0.0-6.1%) was not judged a health risk in any subject. Mild, reversible side effects included constipation (n=2), transient fatigue (n=5), and leg cramps (n=2). Among nine patients with rapid pre-trial progressive disease (PD) five demonstrated post-trial SD or partial remission (SD/ PR) on PET. SD/PR correlated with three-fold higher ketosis compared to those with continued PD (n=4), ($p < 0.02$), but was uncorrelated with reduced calorie intake ($p = 0.45$) or weight loss ($p = 0.81$). Insulin correlated inversely with ketosis ($r = 0.62$, $p = 0.026$), but did not correlate with IFG(1 or 2).

Conclusions: Preliminary pilot data in ten subjects demonstrated that an INSINH diet is safe and feasible in selected patients with advanced cancer. The extent of ketosis, but neither calorie deficit nor weight loss correlated with SD/PR. The small sample size requires cautious interpretation. Further evaluation is needed to explore the relation of insulin inhibition to calorie restriction, as well as a potential therapeutic role of diet adjunctive to metabolic or cytotoxic therapies.

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P61

p53, a novel regulator of lipid metabolism pathways

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Background: In this study we aimed at characterizing the regulation of hepatic metabolic pathways by the p53 transcription factor.

Materials and methods: Analysis of gene expression following alteration of p53 status in several human- and mouse-derived cells using microarray analysis, quantitative real-time PCR, chromatin immunoprecipitation and reporter gene assays. A functional assay was performed to determine lipid transfer activity.

Results: We identified a novel role for the p53 protein in regulating lipid and lipoprotein metabolism, a process not yet conceived as related to p53, which is known mainly in its tumor suppressive

functions. We revealed a group of 341 genes whose expression was induced by p53 in the liver-derived cell line HepG2. Twenty of these genes encode proteins involved in many aspects of lipid homeostasis. The mode of regulation of three representative genes (*pltp*, *abca12* and *cel*) was further characterized. In addition to HepG2, the genes were induced following activation of p53 in human primary hepatic cells isolated from liver donors. p53-dependent regulation of these genes was evident in other cell types namely Hep3B cells and mouse hepatocytes. Furthermore, p53 was found to bind to the genes' promoters in designated p53 responsive elements and thereby increase its transcription. Importantly, p53 augmented the activity of secreted PLTP, which plays a major role in lipoprotein biology and atherosclerosis pathology. **Conclusions:** These findings expose another facet of p53 functions unrelated to tumor suppression and render it a novel regulator of hepatic lipid metabolism and consequently of systemic lipid homeostasis and atherosclerosis development.

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Glucose metabolism is linked to the inflammatory status of macrophages

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Background: Macrophages infiltrate adipose tissue at the onset of weight gain and directly contribute to adipose inflammation, insulin resistance, and obesity [1]. The type of fuel substrate utilized by macrophages is central to the formation of obesity, a global epidemic [2]. Our goal is to understand the role of macrophage glucose metabolism in the promotion of inflammation and insulin resistance during high fat diet-induced obesity. We hypothesize that macrophages with *blunted or elevated* glucose metabolism will display *limited or exaggerated* immune responses, and modulate susceptibility to insulin resistance and obesity, respectively.

Materials and methods: GLUT1 is the glucose transporter expressed by macrophages [3]. We manipulated macrophage glucose metabolism using GLUT1 over-expression and deletion techniques *in vitro*, *ex vivo*, and *in vivo*. *In vitro* studies involved over-expression of GLUT1 in RAW264.7 cells. A high fat diet-induced obesity model involving a novel macrophage-specific *Glut1* knockout mouse (*Glut1M Φ ^{-/-}*) was used to assess total body weight, glucose tolerance, tissue histological alterations, and gene expression changes resulting from *Glut1* deletion. Bone marrow-derived macrophages (BMDMs), isolated from *Glut1M Φ ^{-/-}* mice fed a control diet, were used for measures of polarization, and glucose uptake and metabolism.

Results: GLUT1 over-expression resulted in elevated glucose uptake and metabolism, as well as a hyper-inflammatory state characterized by elevated secretion of MCP-1 and PAI-1, all of which could be blunted by a pharmacologic inhibitor of glycolysis. Preliminary data suggests that *Glut1M Φ ^{-/-}* mice fed a high fat diet were resistant to obesity, remained normoglycemic and demonstrated blunted inflammation in liver and adipose. *Glut1M Φ ^{-/-}* BMDMs were viable, but metabolized less glucose at baseline and after LPS stimulation.

Conclusions: The capacity to use glucose as a fuel is correlated to the inflammatory status of macrophages which likely plays an integral role in the promotion of obesity-related insulin resistance. Possible mechanisms linking glucose metabolism to inflammation are being investigated. Understanding macrophage glucose metabolism and inflammation will identify metabolic and/or signaling pathways that will serve as novel therapeutic targets in the treatment of diabetes and obesity.

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P63

Preoperative fasting protects aged-corpulent mice against renal ischemia-reperfusion injury

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Background: Oxidative stress (OS), the production of free oxygen radicals caused by for instance renal ischemia/reperfusion injury (IRI), results in age associated diseases and accelerated aging. We have shown that preoperative fasting in young-lean C57BL6 male mice protects against renal IRI [1]. Since human patients are usually older and suffer from (co)morbidities, we investigated the effects of preoperative fasting on OS induced by renal IRI in both female and male aged-corpulent mice in a F1-FVB/C57BL6-hybrid background.

Materials and methods: Male and female wild type littermates with an initial age of 72 weeks and average weight of 47.4 and 47.1 grams respectively were preoperatively giving either normal chow or were fasted for 72 hours followed by renal IRI. In the males, IRI was induced by clamping both renal pedicles for 37 minutes. In females, two ischemia times were applied: 37 and 60 minutes. Survival, body weight and wellbeing of the animals were monitored until day 28 postoperatively. P-values of <0.05 were considered significant.

Results: Survival of male mice after 37 minutes of renal IRI was significantly better after 72 hours of fasting in comparison with the ad libitum fed mice (p=0.0171). All female mice survived in the first week after 37 minutes of IRI. When extended to 60 minutes of IRI, a significant difference between the two groups could be seen in favor of the fasted mice (p=0.0040). Body weight decreased in the first two weeks with normalization in the subsequent two weeks.

Conclusions: Similar to young healthy male mice, preoperative fasting induces protection against renal IRI in both male and female aged mice. Old mice have a slower recovery of their body weight after surgery. These results suggest a general protective effect of dietary restriction against renal IRI, regardless of age, sex, body mass and genetic background. Therefore it may be applicable in older patients as well.

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Ethanol and acetaldehyde mediate folic acid and human papillomavirus-induced proliferation of oral squamous cell carcinoma cells *in vitro*

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Background: Although great scientific emphasis has been placed upon HPV as the primary cause of cervical cancers and its involvement in carcinogenic progression of other cancers, less attention has been

focused on the secondary factors that are associated with progression from subclinical HPV infection to invasive carcinoma. Among the secondary factors that limit virus production and carcinogenic progression is CpG methylation of the HPV genome. Several studies now confirm that CpG site-specific methylation of HPV DNA, mediated in part by folate availability, is sufficient to suppress neoplastic progression. In contrast, demethylation or hypomethylation of HPV-DNA sequences is required for transformation, revealing the importance of preferential DNA methylation at CpG sites in the HPV long control region (LCR) between L1 and E6 HPV genes, in addition to the tumor suppressor sites in p53 exons 248 and 273. Because HPV has the potential to initiate oncogenesis, and also to modulate oral cancer growth and folate plays a central role in mediating the availability of methyl groups for CpG-specific DNA methylation (modulating both p53 and HPV mRNA expression) – an investigation of these inter-connected and inter-related mechanisms in oral cancers must be undertaken. In addition, ethanol and acetaldehyde may also play critical roles in determining folate availability, and are primary risk factors for the development of oral cancers, which makes the evaluation of these interconnected metabolic pathways critically important.

Materials and methods: Using a comprehensive series of integrated *in vitro* assays, including proliferation, viability and mRNA analysis using RT-PCR, distinct effects of ethanol and acetaldehyde administration were observed in the oral cancer cell lines, CAL27, SCC15 and SCC25. In addition, the growth modulating effects of HPV infection and FA supplementation were also examined.

Results: Both high-risk HPV strains 16 and 18 induced robust growth-stimulating effects in CAL27 cells, while strain-specific responses were observed in SCC25 and SCC15 cells. FA administration [0–400 μ g/mL] significantly increased the growth rate of all cell lines evaluated. In addition, FA administration induced broad, general increases in cell viability among all cell lines that were associated with p53 mRNA transcriptional down-regulation. None of these cell lines were found to harbor the common C677T mutation in methylenetetrahydrofolate reductase (MTHFR), which can reduce FA availability and may increase oral cancer risk.

Administration of ethanol or acetaldehyde [10 – 100 μ M] significantly inhibited oral cancer cell growth in all cell lines tested. Moreover, the growth inhibiting properties of ethanol and acetaldehyde also mediated and reduced the HPV- and FA-induced growth of CAL27, SCC15 and SCC25 cells. These changes were associated with a transcriptional up-regulation of alcohol dehydrogenase (ADH) and other enzymes involved in alcohol metabolism.

Conclusions: Although tobacco and alcohol use are the main risk factors for developing oral cancer, HPV infection and FA availability may modulate their growth and progression. This study provides preliminary evidence that alcohol and acetaldehyde may mediate and down-regulate the growth enhancing effects of either HPV or FA *in vitro* and may be associated with up-regulation of metabolic pathways involved with alcohol metabolism.

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The ghrelin-growth hormone axis preserves gluconeogenesis to maintain blood glucose levels during starvation

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Background: The octanoylated peptide ghrelin stimulates growth hormone (GH) secretion during severe calorie restriction in mice, which preserves fasting blood sugar after body fat has been depleted. Genetic deletion of ghrelin-O-acyltransferase (GOAT, which octanoylates ghrelin) renders mice ghrelin-deficient and abrogates the normal rise in GH after several days of calorie restriction, resulting in profound hypoglycemia. Administration of either ghrelin or GH to GOAT knockout mice restores the ability to maintain fasting blood sugar levels during prolonged calorie restriction. The mechanism of hypoglycemia in ghrelin-deficient mice has not been described previously.

Materials and methods: 8-week-old male wild-type (WT) and GOAT knockout mice were individually caged and fed 40% of their average daily food intake at 6pm daily during the period of calorie restriction. For glucose production measurements, 4 days prior to calorie restriction each mouse was implanted with a jugular vein catheter. On day 6 of calorie restriction at 4 pm, glucose production was assessed by infusing [$^3\text{-}^3\text{H}$]glucose through the catheter. Blood was obtained from cut tails during the infusion and plasma was separated by centrifugation and deproteinized. The supernatant was evaporated, resuspended in water, and radioactivity was quantified with liquid scintillation counting.

Results: GOAT and ghrelin knockout mice fail to show the normal rise in GH when subjected to fasting after several days of 60% calorie restriction. Both mutant strains become fat-depleted and develop profound hypoglycemia under these conditions. High fat feeding of GOAT knockout mice prior to calorie-restriction doubled body fat percentage and delayed onset of hypoglycemia from day 6 of calorie restriction to day 12. This was associated with a delayed decline in plasma free fatty acids and β -hydroxybutyrate. Plasma levels of lactate and pyruvate in calorie-restricted GOAT knockout mice were half the levels of WT mice. Fasting hypoglycemia in GOAT knockout mice was associated with a glucose production rate that was only 40% of the rate in WT mice. Administration of lactate, pyruvate, and alanine, which can be used as gluconeogenic precursors, restored blood glucose in GOAT knockout mice. Administration of octanoate, which cannot be used as a gluconeogenic precursor, increased glucose production to the level seen in WT mice and restored blood glucose, presumably by providing energy for gluconeogenesis.

Conclusions: Ghrelin-mediated stimulation of GH is critical for maintaining blood glucose in fasting mice when fat stores have been depleted. GH maintains plasma gluconeogenic precursors and preserves glucose production when endogenous (fat) and exogenous (food) fuel sources are absent. These studies demonstrate that ghrelin and GH allow mice to maintain blood glucose during starvation. Elevated levels of both hormones are also seen in starved humans with anorexia nervosa or kwashiorkor, suggesting that the ghrelin-GH system prolongs life during starvation in both mouse and man.

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Dietary restriction and fasting downregulate complement activity

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Background: Seventy-two hours of preoperative fasting (F) or 2 weeks of 30% dietary restriction (DR) offers robust protection against renal ischemia-reperfusion injury (IRI) in mice. However, the mechanism remains to be elucidated. We hypothesize that immunomodulation plays a pivotal role. Innate immunity, especially the complement system, is crucial in the pathophysiology of IRI. Therefore, we investigated the impact of fasting and dietary restriction on complement activation pathways.

Materials and methods: Male C57Bl/6 mice were fed *ad libitum* (AL) or underwent 72 hours fasting or 30% dietary restriction for 2 weeks (n=8/group). Consequently blood was drawn and serum was aliquoted and stored at -80°C. Functional activity of the complement activation pathways (classical (CP), lectin (LP) and alternative pathway (AP)) was assessed by ELISA using immobilized ligands. Deposition of C3 and C9 as a measure of complement activity along with concentration of upstream complement initiating proteins (MBL-A and -C, and C1q) was determined.

Results: A significant downregulation in CP and LP activity by dietary restriction and in CP, LP and AP activity by fasting was observed, compared to the *ad libitum* group. The activation of both C3 and C9 in the dietary restriction and fasting group was significantly downregulated (p<0.002) in CP, LP and AP (except for C3 activation in the AP of the dietary restriction group). The MBL-A concentrations were significantly lower (p<0.001) after dietary restriction and fasting; 15.4 $\mu\text{g/ml}$ (DR) and 12.4 $\mu\text{g/ml}$ (F) compared to 19.9 $\mu\text{g/ml}$ in *ad*

libitum mice. MBL-C concentrations were also significantly lower (p<0.0001) in the dietary restriction and fasting groups; 89.4 and 49.5 $\mu\text{g/ml}$ respectively compared to 109.6 $\mu\text{g/ml}$ in the *ad libitum* group. C1q concentration was only significantly lower in the fasted group (p<0.0001).

Conclusion: Dietary interventions downregulate complement activation pathways. Compared to dietary restriction, fasting has a more pronounced effect. CP seems to be more affected by dietary restriction while AP is most affected by fasting. To our knowledge, our data for the first time show that DR and fasting cause downregulation of complement activation pathways. Therefore, we conclude that complement downregulation may be one of the mechanisms by which dietary interventions protect against renal IRI.

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Dynamic regulation of adipose tissue metabolism in the domestic broiler chicken – an alternative model for studies of human obesity

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Background: The domestic chicken is an attractive, but underutilized, animal model for studies of adipose tissue biology, metabolism and obesity: 1.) like humans, chickens rely on liver rather than adipose tissue for the majority of *de novo* lipogenesis; 2.) quantitative trait loci (QTLs) linked to fatness in chickens contain genes implicated in human susceptibility to obesity and diabetes; 3.) chickens are naturally hyperglycemic and insulin resistant; and 4.) a broad selection of genetic models exhibiting a range of fatness are available. To date, however, little is known about regulation of adipose metabolism in this model organism.

Materials and methods: Affymetrix arrays were used to profile gene expression in abdominal adipose tissue from broiler chickens fed *ad libitum* or fasted for five hours and from three distinct genetic lines with low (Fayoumi and Leghorn) or high (broiler) levels of adiposity. QPCR was used to validate microarray results for select genes. Western blotting was used to assay levels of signaling proteins. Tissue levels of beta-hydroxybutyrate were measured as an index of fatty acid oxidation using a colorimetric assay. Multiple testing was controlled using q-value. Mixed linear model and multivariate clustering analysis were implemented in SAS. The Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 (<http://david.abcc.ncifcrf.gov/>) was used for Gene Ontology (GO) and KEGG pathway enrichment analyses.

Results: A total of 1780 genes were differentially expressed in fasted vs. *ad libitum* fed (p<0.05) tissue after correction for multiple testing. Gene Ontology and pathway analyses, combined with Western blot validation, indicated significant effects on a broad selection of pathways related to metabolism, stress signaling and adipogenesis. In particular, fasting upregulated rate-limiting genes in both the mitochondrial and peroxisomal pathways of beta-oxidation. Enhanced fatty acid oxidation in white adipose tissue was further suggested by a significant increase in tissue content of the ketone beta-hydroxybutyrate. Expression profiles suggested that, despite the relatively brief duration of feed withdrawal, fasting suppressed adipogenesis; expression of key genes in multiple steps of adipogenesis, including lineage commitment from mesenchymal stem cells, were significantly down-regulated in fasted vs. fed adipose tissue. Interestingly, fasting increased expression of several inflammatory adipokines and components of the toll-like receptor 4 signaling pathway. Microarray analysis of Fayoumi, Leghorn and broiler adipose tissue revealed that genetic leanness shared molecular signatures with the effects of fasting. In supervised clustering analysis, fasted broiler chickens clustered with lean Fayoumi and Leghorn lines rather than with the fed broiler group, suggesting that fasting manipulated expression profiles to resemble those of the lean phenotype.

Conclusions: Collectively, these data suggest that leanness in chickens is associated with increased fat utilization which, given the similarities between avian and human adipose tissue with regard to lipid metabolism, may have relevance for humans. The paradoxical increase in some inflammatory markers with an acute fast suggests that the dynamic relationship between inflammation and adipose metabolism may differ from what is observed in obesity. These results highlight chicken as a useful model in which to study the interrelationships between food intake, adipose development, metabolism, and cell stress.

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Flux analysis of nutrient substrates in living cells: human glioblastoma cells partially decouple the TCA cycle from oxidative phosphorylation associated with rapid cell proliferation

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Cancer cells, unlike normal cells, show selective dependency on certain nutrient substrates. These cells exhibit an addiction to glucose or glutamine, and a lack of flexibility in using a full spectrum of nutrient substrates. This dependency varies widely among different cancer cell types and depends on specific mutations in oncogenes and tumor suppressors, the cellular context in which these mutations occur and epigenetic factors. These wide variations in metabolic states have implications for cancer cell responses to therapy.

To date, methods to analyze cellular substrate metabolism have required either the use of radioactive or stable isotope labeled substrate and/or large quantity of cells, may be labor intensive and have low throughput. Stable isotope labeling coupled with Mass Spectrum and NMR analysis has provided highly detailed information about substrate metabolism, but is relatively inaccessible techniques for a majority of researchers and they require special skills and expertise. We have devised several alternative easier and more rapid experimental methods to analyze substrate flux and metabolic states of cancer cells in a microplate. They have allowed us to analyze substrate flux including glucose, glutamine, and fatty acids, and to interrogate

their bioenergetic machineries, glycolysis and mitochondrial oxidative phosphorylation. These methods measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of living cells, and can determine their dynamic responses to pathway perturbations in real-time allowing substrate flux analysis. Essentially, a large number of cell types (lines) can be analyzed using only small quantities of materials and over a short time periods (1-3 hrs) in a microplate using the XF Extracellular Flux analyzer.

In proof-of-principle experiments, we investigated substrate flux and the metabolic state of a syngenic pair of human glioblastoma cells, SF188s (slowly dividing) and SF188f (fast dividing) cells. We found that SF188f cells exhibited a substrate shift, away from glycolysis toward glutamine and fatty acid oxidation, in contrast to SF188s cells. The mitochondrial respiratory capacity and basal respiration of SF188f cells were markedly increased, but the ATP production-coupled respiration was greatly reduced. Further, the leaked respiration was also increased compared with the SF188s cells. That is, a large fraction of SF188f cells' mitochondrial respiration was not devoted to ATP production. We suggest that cancer cells can decouple the TCA cycle from oxidative phosphorylation on an "on demand" basis. This decoupling provides a mechanism by which cancer cells can overcome the metabolic control imposed on the TCA cycle by oxidative phosphorylation, enabling them to meet their extra demand for building block synthesis in addition to energy. This also provides an explanation for the excessive rate of oxygen consumption observed in SF188f cells. In these studies, we also demonstrated that the metabolic state of SF188s and SF188f cells, e.g., glycolysis relative to glutamine oxidation, correlates with their selective susceptibility to inhibitors of glycolysis, 2-deoxyglucose, and glutamine oxidation, aminooxyacetate respectively. Therefore, these methods provide valuable tools for rapid analysis of substrate flux and bioenergetic machineries in cancer or non-cancer cells. They can also enhance understanding the genetic and epigenetic regulation of cancer metabolism in cancer development.

Reference

1. Wu M, Neilson A, Swift A L, Moran R, Tamagnine J, Parslow D, Armistead S, Lemire K, Orrell J, Teich J, Chomicz S, Ferrick DA: Multiparameter metabolic analysis reveals a close link between attenuated mitochondrial bioenergetic function and enhanced glycolysis dependency in human tumor cells. *Am J Physiol Cell Physiol* 2007, **292**:C125-C136.