Research Advances at the Institute for Nutritional Sciences at Shanghai, China

Yan Chen,* Xu Lin, Yong Liu, Dong Xie, Jing Fang, Yingying Le, Zunji Ke, Qiwei Zhai, Hui Wang, Feifan Guo, Fudi Wang, and Yi Liu
Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

ABSTRACT
Nutrition-related health issues have emerged as a major threat to public health since the rebirth of the economy in China starting in the 1980s. To meet this challenge, the Chinese Academy of Sciences established the Institute for Nutritional Sciences (INS) at Shanghai, China ~8 y ago. The mission of the INS is to apply modern technologies and concepts in nutritional research to understand the molecular mechanism and provide means of intervention in the combat against nutrition-related diseases, including type 2 diabetes, metabolic syndrome, obesity, cardiovascular diseases, and many types of cancers. Through diligent and orchestrated efforts by INS scientists, graduate students, and research staff in the past few years, the INS has become the leading institution in China in the areas of basic nutritional research and metabolic regulation. Scientists at the INS have made important progress in many areas, including the characterization of genetic and nutritional properties of the Chinese population, metabolic control associated with nutrient sensing, molecular mechanisms underlying glucose and lipid metabolism, regulation of metabolism by adipokines and inflammatory pathways, disease intervention using functional foods or extracts of Chinese herbs, and many biological studies related to carcinogenesis. The INS will continue its efforts in understanding the optimal nutritional needs for Chinese people and the molecular causes associated with metabolic diseases, thus paving the way for effective and individualized intervention in the future. This review highlights the major research endeavors undertaken by INS scientists in recent years. Adv. Nutr. 2: 428–439, 2011.

Introduction

The INS2 was founded on December 15, 2003 by the Chinese Academy of Sciences. The INS is located on the campus of Shanghai Institutes for Biological Sciences, Shanghai. The mission of the INS is to serve the national interest in promoting health and combating nutrition-related diseases that have emerged as a major threat to public health during the rapid economic growth in the past decades. The rapid changes in lifestyle, dietary patterns, and economic wealth in China have witnessed a substantial shift in disease patterns. Nutrition-related diseases such as type 2 diabetes, metabolic syndrome, obesity, cardiovascular diseases, and many types of cancers have been escalating in the past 30 y in China. Currently, there are ~90 million people suffering from type 2 diabetes in China, with over 150 million people being prediabetic (1). The INS is challenging the frontiers of nutrition-related diseases through multidisciplinary biomedical research at the molecular, cellular, animal, and human population levels, with a long-term focus on disease prevention and intervention.

The INS currently has a total of 25 research groups with orchestrated studies in the areas of metabolic diseases, cardiovascular diseases, and cancers. All the principal investigators were recruited from overseas with solid training in nutrition, medicine, and biomedical research. In addition to 25 primary faculty members, the INS has a total of ~130 research and administrative staff. Training of graduate students at the Ph.D. level has been a major task of the INS, with ~25 Ph.D. students graduating annually since 2008. Due to the space limitation in this review, we will only introduce the research performed by scientists at the...
INS without citing the research performed by many other scientists in related areas.

**Nutrition and genetics in the Chinese population**

One of the major efforts in Dr. Xu Lin’s laboratory at the INS is to establish a population-based cohort study entitled “Nutrition and Health of Aging Population in China” to investigate the effects of environmental and genetic factors and their interaction on the development of metabolic diseases. The baseline survey was conducted in 2005 and a total of 3289 men and women aged 50–70 y were recruited from urban and rural areas of Beijing and Shanghai. The follow-up study with this population is ongoing in 2011 and the information including socio-demographic variables, health status, diet/lifestyle, and mental health will be collected again via a home interview. Anthropometric measurements and biological samples (blood and urine) will also be performed as in the baseline study. Moreover, Dr. Lin’s group also conducted an obesity case-control project in 2007 to investigate the specific roles of gut microbiota and interactions between gut microbiota, nutrition, and genetic factors on glucose and lipid metabolisms, inflammatory status, and metabolic outcomes. The major findings from these observational epidemiologic studies are as follows.

**Genetic factors**

Dr. Lin’s group found that the common variants in CDKAL1, CDKN2A/B, IGF2BP2, SLC30A8 GCKR, and KCNQ1 genes were independently or additively associated with the risk of type 2 diabetes (2) but could not replicate the previously reported associations between FTO variant and the risks of obesity and type 2 diabetes in this population-based cohort of Chinese Hans (2). Different genetic architecture and allele frequencies between Chinese Hans and white Europeans may explain the discrepancy between these data and previous findings from European populations. Remarkably, the common variants at CDKAL1 and KCNQ1 loci showed stronger effect sizes in Chinese Hans than in white Europeans and the joint effects of the 17 diabetes-associated variants, as measured by genetic risk score, could significantly improve discrimination for type 2 diabetes beyond the conventional risk factors (3). In addition, common variants in or near FGF5, CYP17A1, and MTHFR were significantly associated with hypertension risk and the effect sizes of these 3 loci tended to be larger in Chinese than in white Europeans (4). Genetic variation of vitamin D and iron pathway genes were also significantly associated with circulating 25-hydroxyvitamin D and ferritin levels, respectively (X. Lin, unpublished data). Finally, Dr. Lin’s group is currently conducting a genome-wide association of type 2 diabetes in Chinese Hans and the discovery stage recently was completed.

**Environmental factors**

It was found that ~70% of the study participants had vitamin D deficiency [25(OH)D < 50 nmol/L] or insufficienty [50 ≤ 25(OH)D < 75 nmol/L]. The plasma 25(OH)D level was inversely associated with increased risk of metabolic syndrome and insulin resistance (5). Meanwhile, elevated ferritin level was associated with a high risk of diabetes independent of inflammatory markers, adipokines, and metabolic syndrome (6). Habitual soy protein intake may have a gender-dependent effect on the risk of metabolic syndrome (7). Being physically active was associated with a lower risk of having metabolic syndrome and a better profile of inflammatory factors and adipokines (8). Moreover, the north-south difference in the prevalence of depression was also evidenced and the depression syndrome was significantly associated with insulin resistance but not with inflammatory factors and adipokines (9–11). Recently, Dr. Lin’s laboratory also measured erythrocyte fatty acid composition by GC and a total of 28 type fatty acids, including 3 trans, 4 (n-3) fatty acids, 8 (n-6 fatty acids, 7 MUFA, and 6 SFA were detected in 3258 Beijing and Shanghai residents. A unique pattern of fatty acids was observed in this population. For sample, the levels of trans fatty acids in middle-aged and elderly Chinese were relatively low and were strongly associated with dairy consumption. More analyses are currently underway to evaluate the specific effects of different fatty acids on metabolic outcomes.

**Nutritional intervention**

Besides observational studies, Dr. Lin’s group conducted nutritional intervention trials among individuals with type 2 diabetes or metabolic syndrome. In a randomized, double blind, placebo-controlled, crossover trial, flaxseed lignan supplement significantly improved glycemic control as measured by HbA1c and inhibited the C-reactive protein (CRP) level in type 2 diabetes patients (12,13). In a 3-arm, randomized, controlled trial, a low-intensity lifestyle education program was effective in metabolic syndrome management. Flaxseed and walnut supplementation significantly increased the reversion rate of central obesity compared with those who received the lifestyle education program alone (14). In addition, a meta-analysis of 28 randomized, controlled trials of flaxseed or its derivatives on lipid profiles was also conducted and it was found that interventions with flaxseed or lignan, but not flaxseed oil, significantly reduced circulating total and LDL cholesterol (15). In another randomized intervention study, replacing white rice with brown rice for 16 wk showed no substantial improvement for metabolic risk factors, although the participants had a more significant reduction in their diastolic blood pressure compared to those in the white rice arm (X. Lin, unpublished data).

**Nutrient sensing and regulation of metabolism**

**Amino acid sensing and metabolism**

Nutrients such as amino acids are sensed by proteins in cell plasma membrane of cells and they induce a cascade to intracellular signaling to maintain a balance in metabolic status. Dr. Feifan Guo’s laboratory at the INS has extensively investigated how leucine sensing is coupled to the regulation of energy metabolism. Previous studies by Dr. Guo have revealed that leucine deficiency for 7 d inhibits lipogenesis in...
the liver by decreasing sterol regulatory element binding protein 1c and fatty acid synthase expression in a GCN2-dependent manner (16). In addition, the leucine-deprived mice experienced a dramatic reduction in abdominal fat mass (16). Recent studies in Dr. Guo’s laboratory suggest that the fat loss upon leucine deprivation is caused by increased energy expenditure, increased utilization and decreased synthesis of fatty acids in white adipose tissue, and increased thermogenesis in brown adipose tissue (17). In addition to changes in fat mass, it was found that the serum insulin level decreased to 3-fold, whereas the blood glucose level remained normal in leucine-deprived mice (16), suggesting increased insulin sensitivity. This observation was confirmed by conducting glucose tolerance tests and insulin tolerance tests under both normal and insulin-resistant conditions (18). Furthermore, it was found that leucine deprivation improves hepatic insulin sensitivity by sequentially activating GCN2 and decreasing mammalian target of rapamycin/ribosomal protein S6 kinase 1 signaling as well as activation of AMPK (18). In addition, Dr. Guo’s laboratory is also interested in exploring novel functions of certain proteins implicated in amino acid regulatory pathways, such as ATF4. A recent study in Dr. Guo’s laboratory has led to identification of a novel function for ATF4 in regulating lipid metabolism and thermogenesis (19). It was found that the Atf4-deficient mice are lean accompanied with an increase in energy expenditure due to increased thermogenesis in brown adipose tissue as well as a decrease in the synthesis of fatty acids in white adipose tissue (19).

**Resveratrol, protein acetylation, and insulin resistance**

Dr. Qiwei Zhai’s laboratory at the INS has made a number of important discoveries about how protein modification is implicated in the regulation of insulin resistance. They found that SIRT1, an NAD-dependent protein deacetylase, improves insulin sensitivity by repressing PTP1B under insulin resistance conditions (20). Resveratrol, a SIRT1 activator, enhanced insulin sensitivity in vitro in a SIRT1-dependent manner and attenuated insulin resistance in mice fed a HFD (20). Moreover, they identified a resveratrol structural analogue, combretastatin A-4, as an activator of AMPK to improve glucose metabolism in db/db mice (21). They also showed that CCAAT-enhancer-binding protein α binds to the promoter region of SIRT1 and upregulates SIRT1 expression during adipogenesis (22). In addition, Dr. Zhai’s laboratory identified that SIRT1 is able to be localized in the cytoplasm and that cytoplasm-localized SIRT1 may enhance apoptosis (23). They recently found that nicotinamide phosphoribosyltransferase could protect against ischemic stroke through a SIRT1-dependent AMPK pathway (24). In addition, their recent studies have revealed a novel class of antagonists for the FFA receptor G protein-coupled receptor 40 and a pair of windmill-shaped enantiomers from Lindera aggregata possessing an ability to improve insulin sensitivity (25,26). During the studies on protein acetyltransferases, Dr. Zhai’s laboratory found that p300/CBP-associated factor acetylates β-catenin and improves its stability (27) and identified a novel protein acetyltransferase Patt1 that is highly expressed in the liver and enhances apoptosis of hepatoma cells (28).

**Vitamin B-1 in neuron death**

Vitamin B-1, also called thiamine or thiamin, helps the body to convert glucose into energy and aids in the function of the brain. Thiamine deficiency (TD) induces regionally selective neuronal death in the brains of humans and animals. It was found that TD-induced neuronal loss is accompanied by a mild and chronic impairment of oxidative metabolism as well as inflammatory responses and glial activation (29). Selective cell death, inflammation, glial activation, and abnormalities in oxidative metabolism are common in many aging-related neurodegenerative diseases, such as Alzheimer’s disease, which is characterized by severe memory loss, cholinergic deficits, and selective cell death in specific brain regions (30). Dr. Zunji Ke’s laboratory at the INS focused on elucidating the mechanisms underlying TD-induced neurodegeneration. They demonstrated that TD upregulated several markers of ER stress, accompanied by ultrastructural abnormality in ER structure. A selective inhibitor of caspase-12 significantly alleviated TD-induced neuronal death (31). In addition, they found that a selective inhibitor of double-stranded, RNA-activated protein kinase not only protected neurons against TD toxicity but also abolished ethanol potentiation on TD-induced neuronal loss. Therefore, RNA-activated protein kinase may function as a convergent protein that mediates the interaction between TD and ethanol (32,33). TD selectively induced neuronal expression of monocyte chemotactic protein-1 (MCP-1) in the neuronal death region in the brain prior to microglia activation and neurodegeneration. Blocking MCP-1 could inhibit TD-induced microglia activation and neuronal death (34). They also revealed that ADAR2-mediated Q/R editing of GluR2 plays an important role in TD-induced neuronal damage (35). Lately, Dr. Ke’s group found that TD could enhance Aβ generation by promoting β-secretase activity and the accumulation of Aβ subsequently exacerbates TD-induced oxidative stress, indicating that thiamine may be used as a therapeutic regent for neurogenerative diseases (36).

**Iron metabolism**

Iron is an important cofactor and nutrient required for a number of essential cell functions. Dr. Fudi Wang’s laboratory at the INS has been studying various aspects of iron metabolism. Systemic iron requirements are met predominantly through the recycling of iron from senescent erythrocytes by macrophages, a process in which the iron exporter ferroportin (Fpn1) is considered to be essential. They activated Fpn1 in macrophages by crossing Fpn1-floxed animals with macrophage-targeted LysM-Cre or F4/80-Cre transgenic mice. Macrophage Fpn1 deletion mice were overtly normal; however, they displayed a mild anemia and iron accumulation in splenic, hepatic, and bone marrow macrophages when fed a standard diet. Iron loading was exacerbated following the administration of iron dextran or...
phenylhydrazine. When \( Fpn1^{-/}\) mice were challenged with an iron-deficient diet, they developed a more severe anemia and strikingly higher splenic iron levels than control mice, indicating significantly impaired iron mobilization from macrophages. Because immune responses can be altered by modulating iron status, they also examined the expression of proinflammatory cytokoty receptors. We found that the expression levels of TNF\(\alpha\) and IL-6 were significantly enhanced in \( Fpn1^{-/}\) macrophages lacking \( Fpn1\). These studies, therefore, demonstrate that \( Fpn1\) plays important roles in macrophage iron release in vivo and in modulating innate immune responses (37). In a collaborative study, they found that TRPML1 (mucolipin 1, also known as MCOLN1) functions as a Fe(2+) permeable channel in late endosomes and lysosomes (38). Such study also indicates that impaired iron transport may contribute to both hematological and degenerative symptoms in patients with mucolipidosis type IV disease (38).

**Molecular mechanisms underlying regulation of metabolism**

**ER stress response in metabolic regulation**

IRE1\(\alpha\) is a key transducer of the unfolded protein response. Autophosphorylation of IRE1\(\alpha\) is required for its activation, which elicits the cellular unfolded protein response and is functionally connected with insulin biosynthesis in pancreatic \( \beta \) cells. Dr. Yong Liu's laboratory at the INS recently found that the scaffold protein RACK1 interacted with IRE1\(\alpha\) in a glucose-stimulated or ER stress-responsive manner in pancreatic \( \beta \) cells and primary islets (39). RACK1 mediates the glucose-inducible assembly of a complex containing IRE1\(\alpha\), RACK1, and PP2A to promote dephosphorylation of IRE1\(\alpha\) by PP2A, thereby inhibiting glucose-stimulated IRE1\(\alpha\) activation and attenuating IRE1\(\alpha\)-dependent increase in insulin production. Moreover, IRE1\(\alpha\) activation is increased and RACK1 abundance is decreased in a mouse model of diabetes. These findings demonstrate that RACK1 functions as a key component in regulating the IRE1\(\alpha\) signaling pathway in pancreatic \( \beta \) cells and provide new and interesting insights into the differential regulation of IRE1\(\alpha\) during early \( \beta \) cell adaptation to high glucose concentrations or in the course of severe ER stress (39).

**SH2B in metabolism**

SH2B1 is a key regulator of body weight in mammals. Dr. Yong Liu’s laboratory recently identified dSH2B as the Drosophila homolog of SH2B1 (40). Their studies reveal that dSH2B binds to Chico and directly promotes insulin-like signaling. Disruption of dSH2B decreases insulin-like signaling and somatic growth in flies. dSH2B deficiency also elevates hemolymph carbohydrate levels, whole-body lipid levels, lifespan, and resistance to starvation and oxidative stress. On the other hand, systemic overexpression of dSH2B results in opposite phenotypes. dSH2B overexpression in fat body decreases lipid and glucose levels, whereas neuron-specific overexpression of dSH2B reduces oxidative resistance and lifespan. Genetic deletion of SH2B1 also results in growth retardation, obesity, and type 2 diabetes in mice. Surprisingly, lifespan and oxidative resistance are reduced in SH2B1 null mice. These data suggest that dSH2B regulation of insulin-like signaling, growth, and metabolism is conserved in SH2B1, whereas dSH2B regulation of oxidative stress and longevity may be conserved in other SH2B family members (40).

**RNA editing in metabolism**

RNA editing via the conversion of adenosine (A) to inosine (I) is catalyzed by 2 major families of ADAR, ADAR1, and ADAR2. This genetic recoding process is known to play essential roles in the brain, due in part to changes in functional activities of edited neurotransmitter receptors and ion channels. Little is known, however, about the physiological regulation and function of A to I RNA editing in peripheral tissues and other biological processes (41). Dr. Yong Liu’s group reported that both ADAR1 and ADAR2 are expressed in the murine pancreatic islets, and ADAR2 is primarily localized in the islet endocrine cells. In contrast to ADAR1, ADAR2 transcripts in the pancreatic islets exhibit a nearly 2-fold increase in insulin-resistant mice chronically fed a HFD. They also show that in pancreatic \( \beta \)-cells not only the expression of ADAR2 but also the glutamate receptor subunit B editing and ADAR2 self-editing are markedly augmented in response to glucose at the physiological concentration for stimulating insulin secretion. Thus, RNA editing by ADAR2 in pancreatic islets and \( \beta \)-cells is metabolically regulated by nutritional and energy status, suggesting that A to I RNA editing is most likely involved in the modulation of pancreatic islet and \( \beta \)-cell function (41). They also studied the effects of ADAR2 knockdown on regulated exocytosis. Indeed, selective knockdown of ADAR2 expression markedly impairs glucose-stimulated insulin secretion in the rat insulinoma INS-1 cells and primary pancreatic islets and significantly diminishes KCl-stimulated secretion of exogenous human growth hormone or endogenous chromogranin B protein in the rat adrenal pheochromocytoma PC12 cells. Interestingly, the secretory defects resulting from ADAR2 deficiency are coupled to decreased expression of Munc18-1 and synaptotagmin-7, 2 key molecules in the regulation of vesicle exocytosis. Thus, these findings reveal an important role of ADAR2 in regulated exocytosis, implicating RNA editing in the control of cellular secretory machinery (42).

**Hepatic regulation of metabolic pathways**

**Glucogenogenesis and circadian cycle**

During fasting, mammals maintain glucose homeostasis mainly by glucagon-stimulated hepatic gluconeogenesis. Through the AMP-PKA pathway, glucagon activates one of the key gluconeogenic transcription complexes: cAMP response element-binding protein (CREB) and CRTC2 via triggering their phosphorylation or dephosphorylation, respectively. Although the underlying mechanism is unclear, hepatic glucogenesis is also regulated by the circadian clock, which coordinates glucose metabolism with environmental cues. Circadian regulation of gene expression is achieved by a transcription feedback loop, which is composed of 2 activators, Clock and Bmal1, and 3 repressors: Cry (Cry1
and Cry2), Per (Per1, Per2, and Per3), and Rev-erb-α. Dr. Yi Liu at the INS recently reported that hepatic CREB/CRTC2 activity was modulated by Cry (43). They found that Cry1 expression was elevated at the night-day transition, when it reduced gluconeogenic gene expression by blocking glucagon-mediated increase of intracellular cAMP level and downstream activation of CREB/CRTC2. By biochemical reconstitution studies, they demonstrated that Cry1 inhibited cAMP accumulation by directly interacting with Gαs, the key player in G protein-coupled receptor signaling pathway. As a result, hepatic overexpression of Cry1 not only lowered fasting blood glucose levels but also improved insulin sensitivity in db/db mice, suggesting that compounds that enhance Cry1 activity may provide therapeutic benefit to individuals with type 2 diabetes.

**Hepatic glycogen regulation and postprandial glucose homeostasis**

Most animals experience fasting-feeding cycles throughout their lives. It is well known that the liver plays a central role in regulating glycogen metabolism. However, how hepatic glycogenesis is coordinated with the fasting-feeding cycle to control postprandial glucose homeostasis remains largely unknown. Dr. Van Chen’s laboratory at the INS recently discovered a novel molecular mechanism underlying the coupling of hepatic glycogenesis with the fasting-feeding cycle (44). Through a series of molecular, cellular, and animal studies, they investigated how PPP1R3G, a glycogen-targeting regulatory subunit of PP1, is implicated in regulating hepatic glycogenesis and glucose homeostasis in a manner tightly orchestrated with the fasting-feeding cycle. PPP1R3G in the liver is upregulated during fasting and downregulated after feeding. PPP1R3G associates with glycogen pellet, interacts with the catalytic subunit of PP1, and regulates glycogen synthase activity. In addition, the fasting glucose level is reduced when PPP1R3G is overexpressed in the liver. Hepatic knockdown of PPP1R3G reduces postprandial elevation of glycogen synthase activity, decreases postprandial accumulation of liver glycogen, and decelerates postprandial clearance of blood glucose. However, other glycogen-targeting regulatory subunits of PP1, such as PPP1R3B, PPP1R3C, and PPP1R3D, are downregulated by fasting and increased by feeding in the liver. This study, therefore, indicates that PPP1R3G plays a major role in controlling postprandial glucose homeostasis during the fasting-feeding transition via its regulation on liver glycogenesis (44).

**ACL in fatty liver and diabetes**

ACL is a key lipogenic enzyme that catalyzes the critical reaction linking cellular glucose catabolism and lipogenesis, converting cytosolic citrate to acetyl-CoA. Acetyl-CoA is further converted to malonyl-CoA, the essential precursor for fatty acid biosynthesis. It has yet to be explored whether dysregulation of hepatic ACL is metabolically connected to hepatic steatosis, insulin resistance, and hyperglycemia. Dr. Yong Liu’s laboratory at INS found that in leptin receptor-deficient db/db mice, the expression of ACL is selectively elevated in the liver but not in the white adipose tissue. Liver-specific ACL abrogation via adenovirus-mediated RNA interference prominently reduces the hepatic contents of both acetyl-CoA and malonyl-CoA, markedly inhibits hepatic de novo lipogenesis, and protects against hepatic steatosis in db/db mice. Surprisingly, liver-specific ACL abrogation markedly inhibits the expression of PPARγ and the entire lipogenic program in the liver. Moreover, hepatic ACL deficiency results in significantly downregulated expression of gluconeogenic genes in the liver as well as enhanced insulin sensitivity in the muscle, leading to substantially improved systemic glucose metabolism (45). Meanwhile, they specifically knocked down hepatic ACL expression in wild-type mice maintained on a low-fat or HFD and found that hepatic ACL abrogation markedly reduces the liver abundance of both acetyl-CoA and malonyl-CoA regardless of dietary fat intake, which is paralleled with decreases in circulating levels of TG and FFA. Moreover, hepatic ACL knockout results in diet-dependent changes in the expression of other lipogenic enzymes, accompanied by altered fatty acid compositions in the liver. Together, their findings establish a crucial role of hepatic ACL in lipid and glucose metabolism and indicate that hepatic ACL may serve as a potential target to treat NAFLD and type 2 diabetes (45,46).

**Signaling and regulation of adipokines and cytokines**

**Leptin signaling and energy metabolism**

Leptin is an adipocyte-derived hormone that plays a critical role in energy homeostasis and glucose and lipid metabolism. It regulates energy balance and metabolism by activation of multiple signaling cascades mediated by the long-form leptin receptor Ob-Rb. However, the whole spectrum of signaling actions through the 3 cytoplasmic tyrosines of mouse Ob-Rb remains to be completely defined in vivo. Dr. Yong Liu’s laboratory at the INS generated 3 knockin lines of mice expressing mutant Ob-Rb with phenylalanine substitution for Tyr985 alone (Y985F), Tyr1138 alone (Y3F), or all 3 tyrosines (Y123F) (47). They found that Y123F animals develop overt obesity similar to that of Y3F animals with abrogated hypothalamic activation of STAT3 by leptin, but they exhibit more severe impairment in glucose tolerance. In striking contrast to db/db mice, however, both Y123F and Y3F mice show attenuated adiposity with reduced hyperphagia, marked improvement in physical activity and adaptive thermogenesis, and significantly ameliorated glycemic control. Further, Y123F mice have hypothalamic neuuropeptide Y/agouti-related protein expression maintained at prominently lower levels compared with db/db mice. Their findings provide direct physiological evidence that Ob-Rb exerts crucial metabolic actions not only through tyrosine-dependent but also tyrosine-independent mechanisms in control of energy balance and glucose homeostasis (47). In addition, Dr. Liu’s team found that, surprisingly, although young homozygous Y985F animals are slightly leaner, they exhibit adult onset or diet-induced obesity. Both age-dependent and diet-induced deterioration of energy balance is
paralleled with pronounced leptin resistance, which is largely attributable to attenuation of leptin-responsive hypothalamic STAT3 activation as well as prominently elevated expression of hypothalamic SOCS3, a key negative regulator of leptin signaling. Their findings unmask distinct binary roles for Try985-mediated signaling in energy metabolism, acting as an age-/diet-dependent regulatory switch to counteract age-associated or diet-induced obesity (47,48).

ADIPOR and insulin resistance
Adiponectin is an adipocyte-derived hormone that plays a critical role in the development of type 2 diabetes via interaction with ADIPOR1 and ADIPOR2. Dr. Yan Chen’s laboratory at the INS has investigated the transcriptional regulation of ADIPOR (49). They found that rosiglitazone, a PPAR agonist that is widely used in the treatment of type 2 diabetes, was able to regulate lipid and glucose metabolism through modulation of the expression of ADIPOR2. They found that rosiglitazone elevated the mRNA and protein levels of ADIPOR2 and stimulated ADIPOR2 promoter in HepG2 cells. Analysis with the ADIPOR2 promoter revealed a putative rosiglitazone-responsive region that contained a glucocorticoid receptor-binding element. In addition, treatment of mice with rosiglitazone elevated the expression of ADIPOR2 in the liver. This study, therefore, suggests that the PPARγ agonist rosiglitazone can functionally interact with a glucocorticoid receptor element in the ADIPOR2 promoter to mediate stimulation of transcription, revealing a new paradigm underlying the therapeutic effect of PPARγ activators in the treatment of type 2 diabetes (49). Furthermore, Dr. Chen’s laboratory has identified the molecular mechanism implicated in transcriptional regulation of ADIPOR1 by insulin (50). They found that a nuclear inhibitory protein binding element at the promoter of ADIPOR1 gene is involved in the negative regulation of AdipoR1 promoter by insulin.

They also analyzed endocytosis of adiponectin and AdipoR1 and found that both are internalized into transferrin-positive compartments that follow similar traffic routes (51). Blocking clathrin-mediated endocytosis by expressing Eps15 mutants or depleting K(+) trapped AdipoR1 at the plasma membrane, and K(+) depletion abolished adiponectin internalization, indicating that the endocytosis of AdipoR1 and adiponectin is clathrin dependent. These data indicate that AdipoR1 is internalized through a clathrin- and Rab5-dependent pathway and that endocytosis may play a role in the regulation of adiponectin signaling (51).

apo-AI in obesity and insulin
In humans, one of the hallmarks of type 2 diabetes is a reduced plasma concentration of HDL and its major protein component, apoA-I (APOA-I). However, it is unknown whether APOA-I directly protects against diabetes. Dr. Yan Chen’s laboratory has characterized the functional role of APOA-I in glucose homeostasis (52). They found that APOA-I was able to stimulate the phosphorylation of AMPK and ACC and elevated glucose uptake in C2C12 myocytes. APOA-I could be endocytosed into C2C12 myotubes through a clathrin-dependent endocytotic process. Inhibition of endocytosis abrogated APOA-I–stimulated AMPK phosphorylation. In Apoa-1 (−/−) mice, AMPK phosphorylation was reduced in skeletal muscle and liver, and expression of gluconeogenic enzymes was increased in liver. In addition, the Apoa-1 (−/−) mice had a higher fat content and compromised glucose tolerance. Therefore, these data indicate that APOA-I has a protective effect against diabetes via activation of AMPK (52). They also analyzed the antiobesity effect of ApoA-I using 2 mouse models, a transgenic mouse with overexpression of ApoA-I and mice administered with an ApoA-I mimetic peptide D-4F. The mice were induced to develop obesity by receiving a HFD. Both ApoA-I overexpression and D-4F treatment could significantly reduce white fat mass and slightly improve insulin sensitivity in the mice. ApoA-I and D-4F treatment was able to increase UCP1 mRNA and protein levels as well as to stimulate AMPK phosphorylation in brown adipocytes in culture. Taken together, these results reveal that ApoA-I has an antiobesity effect in mice and such an effect is associated with increases in energy expenditure and UCP1 expression in brown fat tissue (53).

IL-22 in lipid metabolism
IL-22 is a Th17-related cytokine within the IL-10 family and plays an important role in host defense and inflammatory responses in orchestration with other Th17 cytokines. IL-22 exerts its functions in nonimmune cells as its functional receptor IL-22R1 is restricted in peripheral tissues but not in immune cells. Dr. Yan Chen’s laboratory has identified that IL-22 has an effect on lipid metabolism in the liver (54). They found that IL-22 could alleviate hepatic steatosis induced by a HFD. Administration of rmIL-22 was able to stimulate STAT3 phosphorylation in HepG2 cells and mouse liver. The expression of lipogenesis-related genes, including critical transcription factors and enzymes for lipid synthesis in the liver, was significantly downregulated by IL-22. The levels of TG and cholesterol in the liver were also significantly reduced by long-term treatment of rmIL-22 in C57BL/6 and ob/ob mice fed with HFD. The HFD-induced increases of ALT and AST in ob/ob mice were ameliorated by rmIL-22 treatment. In addition, they found that the expression of fatty acid synthase and TNFα in the liver was decreased by long-term rmIL-22 administration. This study, therefore, indicate that IL-22, in addition to its known functions in host defense and inflammation, has a protective role in HFD-induced hepatic steatosis via its regulation on lipid metabolism in the liver (54).

Chronic inflammation in metabolic regulation

TLR4 in energy metabolism
TLR4 has been reported to induce insulin resistance through inflammation in obese mice. However, the physiological role of TLR4 in metabolism is unknown. Dr. Yingying Le’s laboratory at the INS investigated the involvement of TLR4 in metabolism during fasting (55). They found that mice lacking
the TLR4 gene displayed aggravated fasting hypoglycemia, along with normal hepatic gluconeogenesis but reversed activity of pyruvate dehydrogenase complex in skeletal muscle, which might account for the fasting hypoglycemia. TLR4 (−/−) mice also exhibited higher lipid levels in circulation and skeletal muscle after fasting and reversed expression of lipogenic enzymes in skeletal muscle but not liver and adipose tissue. Lipolysis in adipose tissue is unaffected and fatty acid oxidation in skeletal muscle is increased in TLR4(−/−) mice after fasting. Inhibition of fatty acid synthesis in TLR4(−/−) mice abolished hyperlipidemia, hypoglycemia, and pyruvate dehydrogenase complex activity increase, suggesting that TLR4-dependent inhibition of muscle lipogenesis may contribute to glucose and lipid homeostasis during fasting. Further studies showed that TLR4 deficiency had no effect on insulin signaling and muscle proinflammatory cytokine production in response to fasting. These data suggest that TLR4 plays a critical role in glucose and lipid metabolism independent of insulin during fasting, thus revealing a novel physiological role for TLR4 in fuel homeostasis (55).

**Upregulation of amylin by proinflammatory cytokines**

Amylin, also known as islet amyloid polypeptide, is the major component of amyloid deposits in the pancreas of type 2 diabetes patients. Amylin is mainly expressed and secreted by pancreatic β-cells. Amylin has been reported to be associated with insulin resistance and pancreatic β-cell apoptosis and implicated in the pathogenesis of type 2 diabetes. Dr. Yingying Le’s laboratory examined the effect of proinflammatory cytokines on amylin gene expression. They found that TNFα and MCP-1 induced amylin expression at both mRNA and protein levels in murine pancreatic β-cell line MIN6 and primary islets. Further studies demonstrated that TNFα induced murine amylin expression through PKCζ-Extracellular signal-regulated kinases 1/2/c-Jun N-terminal kinases-activator protein 1 (ERK1/2/JNK-AP1) and P38-nuclear factor-κ-light-chain-enhancer of activated B cells (NF-κB) signaling pathways and enhanced human amylin promoter activation through NF-κB and AP1. MCP-1 induced amylin expression through ERK1/2/JNK-AP1 and NF-κB–related pathways. Collectively, TNFα/MCP-1 induces amylin gene expression in murine pancreatic β-cells through multiple signaling pathways, which may contribute to amylin elevation in inflammation-related pancreatic disorders and their progression (56,57).

**Inflammatory marker and adipokine studies in the Chinese population**

In the project Nutrition and Health of Aging Population in China performed by Dr. Xu Lin’s group at the INS, high plasma CRP, IL-6, retinol binding protein 4, and low adiponectin were found to be independently associated with increased risks of metabolic syndrome and/or type 2 diabetes in the middle-aged and elderly Chinese population (58–61) and the association between resistin and metabolic syndrome seems to be dependent on inflammatory status (62). Notably, the overall CRP concentration in the Chinese population is much lower than that of Americans and nearly 50% of their study participants with metabolic syndrome have a low risk level of CRP according to the criteria of AHA/CDC. More recently, they found that larger leg fat mass, unlike trunk fat depot measured by DXA, is associated with a better profile of adipokines, inflammatory markers, and reduced risk of metabolic syndrome among Chinese (63). Moreover, trunk fat and leg fat also showed opposite associations with plasma ferritin concentrations (63). In their cases-control study, it was found that HMW-adiponectin showed strong inverse associations with metabolic syndrome independent of body composition, inflammation, leptin, and soluble leptin receptor, while the associations of leptin and soluble leptin receptor were largely explained by fat mass or HMW-adiponectin, respectively (64). Elevated plasma IL-18 was associated with higher prevalence of metabolic syndrome, independent of traditional risk factors, fat mass, inflammatory markers, and HMW-adiponectin, but significantly interacted with lean mass (64). It was interesting that data from this study also demonstrated that elevated circulating LPS-binding protein, a marker of subclinical endotoxemia, was associated with obesity, metabolic syndrome, and type 2 diabetes in apparently healthy Chinese (65). These findings suggest a role of LPS via initiation of an innate immune mechanism in metabolic disorders.

**Cancer research at the INS**

**Cancer prevention by natural compounds**

Flavonoids belong to a large group of phenolic plant constituents and they possess bioactive potential. Some of the case-control studies have indicated an inverse association between intake of flavonoids and risk of certain cancers, including lung cancer, upper digestive tract cancer, and gastric cancer. However, the role and molecular mechanisms for inhibition of cancer by these compounds remain largely unknown. Dr. Jing Fang’s laboratory at the INS has determined the effect of some flavonoids such as luteolin, apigenin, and chrysin on tumorogenesis and uncovered the underlying molecular mechanisms. Their studies have revealed that apigenin and chrysin inhibited tumor angiogenesis through attenuating expression of HIFα and VEGF of tumor cells (66,67). In addition, they found that apigenin inhibited tumor metastasis via FAK (68). Their study also indicated that luteolin could induce cancer cell apoptosis through IGF-1/IGF-1R signaling and inhibit cancer metastasis via E-cadherin (69,70). Collectively, these studies suggest that these compounds are potent agents for cancer chemoprevention and/or chemotherapeutics.

**Breast cancer risk and genetic variants**

Dr. Hui Wang’s laboratory at the INS has performed a series of studies to analyze the genetic variation in the Chinese population and the associations with breast cancer susceptibility. They conducted case-control studies to evaluate the association between the variants on PALB2, FGFR2, and TNRC9 and breast cancer susceptibility. They demonstrated that...
variants on PALB2 (rs249954, rs120963, and rs16940342) are significantly associated with breast cancer risk (71). They also reported the variants on FGFR2 (rs2981582, rs1219648, and rs2420946) are statistically associated with breast cancer susceptibility in the Chinese population. Women with 3 risk loci have a 1.36-fold increased risk of breast cancer and the association is even stronger among patients with ER- and PR-positive breast cancer (72). SNPs on TNRC9 (rs3803662, rs12443621, and rs8051542) have been reported to be significantly associated with breast cancer risk in the Caucasian population. However, none of the 3 polymorphisms is found to be significantly associated with breast cancer risk in Chinese population. Only rs12443621 AG/GG genotypes showed an increased risk of ER-positive breast cancer compared with those with homozygous AA genotype (73). These results indicate that the breast cancer in the population may possess unique genetic features. In addition, Dr. Hui Wang’s laboratory has investigated the association of blood vitamin D level with breast cancer. They conducted a meta-analysis to determine the effects of vitamin D intake, calcium intake, and circulating 25(OH)D and 1α,25 (OH)2D levels on breast cancer risk. High vitamin D and calcium intake are associated with a decreased risk of breast cancer and the circulating 25(OH)D level has an inverse relationship with breast cancer, whereas the circulating 1α,25 (OH)2D has no apparent effects on breast cancer risk. The results suggest that vitamin D and calcium have chemopreventive effects against breast cancer (74).

Hypoxia and colorectal cancers
Colorectal cancer is the 3rd most common malignancy worldwide and one of the leading causes of cancer death in the world. The mechanisms of the development of colorectal cancer remain largely unknown. PHD1–3 inhibit expression of HIFα, a key factor in the development of cancer, and are thought to possess tumor suppressor activities. However, the expression and function of PHD in colorectal cancer has yet to be determined. Dr. Jing Fang’s group recently found that the expression of PHD3, but not PHD1 or 2, decreased in colorectal cancer and the decreased PHD3 is associated with higher tumor grade and metastasis (75). Mechanistic studies indicated that, in addition to HIFα, PHD3 also inhibited IKK/NF-κB signaling. Functional investigation demonstrated that knockdown of PHD3 increased tumor growth of colorectal cancer cells. Activation of NF-κB has been commonly observed in human colorectal cancers. They found that PHD3 inhibited IKK/NF-κB signaling. Thus, determination of PHD3 status could aid in targeted therapy for patients with colorectal cancers that have increased NF-κB activity.

Esophageal cancer research
Dr. Dong Xie’s laboratory at the INS discovered the involvement of IRF-1 and IRF-2 in the formation and progression of human esophageal cancers (76). IRF-1 and IRF-2 are generally regarded as a tumor suppressor and an oncoprotein, respectively. However, little is known about their expression and function in ESCC. They found that IRF-1 expression was decreased and IRF-2 expression was increased in ESCC compared with matched normal esophageal tissues. Moreover, statistical data indicated that IRF-2 expression was tightly correlated with progression of ESCC. IRF-1 and IRF-2 are able to regulate tumorigenicity of ESCC cells as antioncoprotein and oncoprotein, respectively. Relative amounts of IRF-1 and IRF-2 are functionally very important for the development and progression of ESCC and reduction of the ratio of IRF-1:IRF-2 may lead to the enhancement of tumorigenicity of ESCC cells. Therefore, levels of IRF-1 and IRF-2 are useful indicators in the diagnosis and prognosis for ESCC and these molecules are potential drug targets for ESCC therapy. Furthermore, their study has revealed a negative feedback regulation of IFNγ pathway by IRF-2 in esophageal cancers (77). IFNγ is an antitumor cytokine that inhibits cell proliferation and induces apoptosis after engagement with the IFNγ receptors expressed on target cells, whereas IRF-2 is able to block the effects of IFNγ by repressing transcription of IFNγ-induced genes. They investigated in detail the functions of IFNγ in esophageal cancer cells. They found that the clinical samples of human esophageal cancers had an increased level of IFNγ that was positively correlated with tumor progression and IRF-2 expression, whereas the level of IFNγ receptor 1 was decreased and negatively correlated with tumor progression and IRF-2 expression. This study disclosed a new IRF-2–mediated inhibitory mechanism for IFNγ-induced pathway in esophageal cancer cells. In addition, their work has found that CTGF is overexpressed in ESCC and promotes tumorigenicity through β-catenin/TCF signaling (78). They found that overexpression of CTGF occurred in a significant proportion of ESCC samples that were of a high tumor grade and metastatic. Forced expression of CTGF in Eca109 ESCC cells accelerated their growth in culture and significantly increased tumor formation in nude mice. Moreover, overexpression of CTGF in ESCC cells resulted in the accumulation and nuclear translocation of β-catenin, leading to activation of β-catenin–TCF/LEF signaling. Furthermore, they identified a β-catenin–TCF/LEF–binding site in the promoter region of CTGF and found that CTGF is a transcriptional target of β-catenin–TCF/LEF signaling. Taken together, these results revealed that the interaction of CTGF and β-catenin–TCF/ LEF forms a positive feedback loop, which could contribute to the tumorigenicity of ESCC.

Liver cancer research
Dr. Dong Xie’s laboratory has been working on the molecular mechanism and therapy of hepatic cancers. They recently discovered that EphrinA2 promotes tumorigenicity through the Rac1/Akt/NF-κB signaling pathway (79). They found that the expression of EphrinA2 was significantly upregulated in both established cell lines and clinical tissue samples of HCC and the most significant increase was observed in the tumors invading the portal veins. They further found that suppression of apoptosis, rather than acceleration of cell proliferation, was responsible for EphrinA2–enhanced tumorigenicity. In addition, EphrinA2 endowed cancer cells
with resistance to TNFα-induced apoptosis, thus facilitating their survival. This study revealed that EphrinA2 plays an important role in the development and progression of HCC by promoting the survival of cancer cells, indicating its role as a potential therapeutic target in HCC. Recently, their research led to a discovery that sorafenib suppresses postsurgical recurrence and metastasis of hepatocellular carcinoma in an orthotopic mouse model (80). They found that sorafenib suppressed the development of postsurgical intrahepatic recurrence and abdominal metastasis and consequently led to prolonged postoperative survival of mice in this model. Furthermore, hyperactivity of extracellular signal-regulated kinase signaling caused by elevated levels of growth factors associated with postoperative liver regeneration enhanced the sensitivity of HCC cells to sorafenib, thus providing a plausible explanation for the observation that recurrent tumors are more responsive to growth inhibition by sorafenib. These results strongly suggest that by effectively reducing postoperative recurrence, sorafenib has a potential application in early-stage HCC patients who have undergone hepatectomy with curative intention.

RKTG, a new tumor suppressor gene
Subcellular compartmentalization has become an important theme in cell signaling. Dr. Yan Chen's laboratory recently discovered that a mode of spatial regulation of Raf kinase by RKTG (Raf kinase trapping to Golgi) or PAQR3 (81). Their studies reveal that RKTG is a 7-transmembrane protein localized at the Golgi apparatus. RKTG expression inhibits EGF-stimulated ERK and RSK phosphorylation and antagonizes Ras- and Raf-1–stimulated Elk-1 transactivation. Through interaction with Raf-1, RKTG changes the localization of Raf-1 from cytoplasm to the Golgi apparatus, blocks EGF-stimulated Raf-1 membrane translocation, and reduces the interaction of Raf-1 with Ras and MEK1. Subsequently, their laboratory discovered that RKTG can also bind and translocate B-Raf to the Golgi apparatus. When overexpressed in A375 human malignant melanoma cells with B-Raf mutation, RKTG inhibits ERK activation, cell proliferation, and transformation of A375 cells. In addition, the tumorigenicity of the RKTG-expressing A375 cells is suppressed in nude mice (82). Using a 2-stage DMBA/TPA carcinosogenesis protocol on mouse skin, the number and size of papillomas are increased in RKTG(−/−) mice, accompanied by shortened tumor latency and enhanced keratinocyte proliferation (83). Recently, Dr. Yan Chen’s group discovered that RKTG is involved in autocrine VEGF signaling required for angiogenesis (84). It was found that RKTG has a negative effect on cell proliferation, migration, sprouting, and angiogenesis of endothelial cells. Furthermore, the expression level of RKTG is significantly downregulated in clinical clear cell renal cell carcinoma tumor samples, with an inverse correlation with VEGF expression level. In addition to the spatial regulation on Raf kinase, RKTG was also identified to regulate signaling of G protein coupled receptors by sequestering Gβ subunit to the Golgi apparatus (85). Interestingly, their recent studies indicate that RKTG has an in vivo tumor suppressor function to cooperate with p53 in tumorigenesis (86). This study also reveals that p53 has an EMT checkpoint function and the loss of this function can combine with loss of RKTG to drive EMT and tumor progression (86). Collectively, these studies suggest that RKTG is a potential tumor suppressor that is implicated in the regulation of a variety of cellular activities, including proliferation, angiogenesis, and EMT.

Chinese herbs and chronic diseases
Natural products in cancer therapy
Dr. Hui Wang’s group at the INS has reported that artemisinin and its derivatives have potent antitumor activities against ovarian and liver cancers, thus providing a basis for future clinical studies using artemisinin compounds as novel therapeutic agents in human cancer chemotherapy (87,88). They have discovered and developed quinazoline-2(1H)-thione derivatives as a novel class of B-cell lymphoma-extra large, B-cell lymphoma 2, and induced myeloid leukemia cell differentiation protein inhibitors (89). They also studied the major active components of ginseng, ginsenosides. They evaluated the inhibitory effects of 15 ginsenosides on human P450 enzymes and explored the substrate-dependent effects and structure-activity relationships in an effort to establish a pharmacophore model. They found that 8 ginsenosides and glycosides potently induced P450 expression and that structure-activity relationships existed for these effects (90,91). Moreover, they recently discovered a novel class of small molecule fluorophores, 2-iminocoumarin-3-carboxamide derivatives, as fluorescent probes that are cell membrane permeable with low cytotoxicity and selectivity and thus can be applicable for mitochondria imaging in living cells (92).

Tan in obesity
PPARγ is a nuclear receptor that coordinates carbohydrate and lipid metabolism and is a therapeutic target for type 2 diabetes. Tan is a lipophilic diterpene that is widely used to treat cardiovascular diseases in traditional Chinese medicine and has recently been found to reduce body weight and lower blood lipids. However, its underlying mechanism of antidiabetic effects remains unknown. Dr. Ying Q. Zhan, a former faculty of the INS, reported that Tan inhibits 3T3-L1 preadipocyte differentiation and transcriptional activities of full-length PPARγ and PPARγ ligand-binding domains (93). They found that the effects of Tan are mediated through its property as a natural antagonist of PPARγ. Tan treatment reduced adipose mass and body weight, improved glucose tolerance, and lowered the LDL:HDL ratio without changing the food intake in a HFD-induced obese animal model. These results suggest that the combined properties of Tan in adipogenesis, glucose tolerance, lipogenesis, and cardiovascular protection are beneficial for treating diabetic patients with complex metabolic conditions, in which modulating a single target is often not sufficient to achieve the desired effect.
Berberine in type 1 diabetes

Berberine, an alkaloid derivative from Berberis vulgaris L., has been used extensively in traditional Chinese medicine to treat diarrhea and diabetes, but the underlying mechanisms for treating diabetes are not fully understood. Because type 1 diabetes is caused by T cell-mediated destruction of β cells and severe islet inflammation, Dr. Ying Q. Zhan’s laboratory recently proposed that berberine could ameliorate type 1 diabetes through its immune regulation properties (94). They found that oral administration of berberine for 2 wk prevented the progression of type 1 diabetes in one-half of the NOD mice and decreased Th17 and Th1 cytokine secretion. Berberine suppressed Th17 and Th1 differentiation by reducing the expression of lineage markers. It was also found that berberine inhibited Th17 differentiation by activating ERK1/2 and inhibited Th1 differentiation by inhibiting p38 MAPK and JNK activation. In addition, berberine downregulated the activity of STAT1 and STAT4 through the suppression of p38 MAPK and JNK activation and it modulated the stability of STAT4 through the ubiquitin-proteasome pathway. These data indicate that berberine targets MAPK to suppress Th17 and Th1 differentiation in type 1 diabetic NOD mice.

Conclusion

In a relatively short time frame, the INS has become the leading institution in China in the areas of nutritional research and metabolic regulation. Scientists at the INS have established a number of vigorous research programs and made considerable progress in many aspects, including characterization of genetic and nutritional properties in Chinese population, metabolic control associated with nutrient sensing, molecular mechanisms underlying glucose and lipid metabolism, regulation of metabolism by adipokines and inflammatory pathways, disease intervention using functional foods or extracts of Chinese herbs, and many biological studies related to carcinogenesis. Given that nutrition-related diseases have currently emerged as the paramount health threat in China in the coming era, the INS will continue its efforts in understanding the molecular causes associated with these diseases and paving the way for effective and individualized intervention in the future.

Literature Cited


