Published in final edited form as:

Int J Sports Med. 2009 September ; 30(9): 631–635. doi:10.1055/s-0029-1224174.

High-Fat Diet Induces IKK β and Reduces Insulin Sensitivity in Rats with Low Running Capacity

B. T. Bikman¹, T. L. Woodlief², R. C. Noland³, S. L. Britton⁴, L. G. Koch⁴, R. M. Lust⁴, G. L. Dohm⁴, and R. N. Cortright⁵

¹ Department of Cardiovascular and Metabolic Diseases, Duke-NUS, Singapore, Singapore

² Department of Physiology, East Carolina University, United States

³ Sarah W. Stedman Nutrition and Metabolism Center, Duke University, United States

⁴ Department of Physical Medicine and Rehabilitation, University of Michigan, Ann Arbor, United States

⁵ Department of Exercise and Sport Science, East Carolina University, United States

Abstract

Rats bred for a high-capacity to run (HCR) do not develop insulin resistance on a high-fat diet (HFD) vs. those bred for a low-capacity for running (LCR). Recently, a link between obesity and insulin resistance has been established via IKK β action and IRS-1 Ser^{312/307} phosphorylation. This study measured I κ B α and IRS-1 pSer³⁰⁷ in mixed gastrocnemius muscle in HCR and LCR rats challenged with a 12-wk HFD. HFD treatment resulted in significantly higher glucose and insulin levels in LCR vs. HCR rats. I κ B α levels, an inverse indicator of IKK β activity, were lower in LCR vs. HCR rats maintained on chow diet and were reduced further following HFD in LCR rats only. IRS-1 pSer³⁰⁷ in the LCR rats increased on the HFD vs. chow. We conclude that differences in glucose tolerance between LCR and HCR rats are at least partly explained by differences in IKK β activity and pSer³⁰⁷ levels.

Keywords

aerobic capacity; IKK β ; insulin signaling; high-fat diet

Introduction

The insulin resistance associated with type 2 diabetes results (at least in part) from a defect in the insulin signaling cascade in a variety of tissues, notably skeletal muscle. Previously, we have shown that muscle glucose transport in obese individuals and type 2 diabetics is reduced and that this is closely linked with an attenuation in insulin-receptor tyrosine kinase activity [16,23]. Whereas tyrosine phosphorylation of insulin-receptor substrate (IRS)-1 is necessary for normal signaling, serine phosphorylation of IRS-1 has been implicated in inhibiting insulin signal transduction [4,22] and the IRS-1 Ser^{312 (Human)/307 (rodent)} residue has received particular interest. In this regard, activation of the NF- κ B pathway is under investigation as it has been suggested as a mechanism that may contribute to the serine phosphorylation of IRS-1 [1,4,22]. Briefly, NF- κ B is held inactive in the cytosol by the inhibitor of κ B α (I κ B α). Upon

activation, a kinase upstream of I κ B α , the inhibitor of κ B kinase (IKK β), phosphorylates I κ B α and is responsible for its rapid degradation. Consequently, NF- κ B is free to migrate into the nucleus and induce transcription of inflammatory cytokines. IKK β also plays an additional role within the cell as a serine kinase where it has been implicated in causing deficient insulin signal transduction through its phosphorylation of IRS-1 Ser³¹² (human)/ 307 (rodent) [1,4,14, 22]. Moreover, IKK β is activated by fatty acid metabolites, which have also been causally linked to insulin resistance as occurs in the obese state [8].

In addition to obesity, several studies have revealed that physical inactivity is an independent risk factor for many of the same diseases associated with obesity [6,12], whereas endurance exercise training is a universally prescribed treatment for most of these diseases. Physical fitness has not only been found to reduce disease risk, but also to lower chronic low-grade inflammation [2,10]. For example, Bogardus et al. [3] reported that low levels of physical activity and poor physical fitness were predictors of all-cause mortality. In support, regular physical activity is associated with positive effects on insulin sensitivity and with a reduced risk of all-cause mortality and type 2 diabetes. In fact, the Kuipio Ischemic Heart Disease Risk Factor Study found that subjects with low physical fitness had more than four times the risk in developing diabetes compared with those of high physical fitness [13]. In a similar study, the adjusted relative risks of developing diabetes when comparing quartiles of physical fitness were 1.0 (normal), 0.78, 0.63, and 0.56 [19].

It is widely accepted that individuals exhibit drastically different aerobic capacities in the absence of a training stimulus and differ vastly in their inherent capacity to adapt to exercise. It is not known whether the genetic components associated with the predisposition to enhanced endurance capacity alone result in protection against metabolic diseases, whether a training stimulus is required, or whether both work synergistically. Recently, a strain of rats has been developed [11] that differ for intrinsic running capacity allowing the role of a genomic predisposition for aerobic exercise capacity to be explored independent of training status. Our lab has previously shown that these rats, bred according to low- and high-capacity endurance running (LCR and HCR, respectively) differ significantly in metabolic function. Specifically, we observed that LCR rats were heavier, hypertriglyceridemic, had reduced muscle oxidative capacity, and were less insulin sensitive compared with HCR rats. Moreover, when fed a high-fat diet (HFD), the LCR rats gained more fat mass and experienced further reductions in glucose tolerance/insulin action, whereas these same outcomes remained unchanged in the HCR rats [16]. The purpose of the current investigation was to determine the potential role of IKK β action and the Ser³⁰⁷ phosphorylation state of IRS-1 which may delineate a mechanism to explain the contrast in insulin sensitivity previously observed between LCR and HCR rats on chow and high-fat diets.

Materials and methods

Animal strains

The development of LCR and HCR rats has been described previously in detail [11]. Briefly, two-way artificial selection of a heterogenous rat population from the N:NIH stock (National Institutes of Health) produced rat strains differing in inherent aerobic capacity. Endurance running capacity was assessed through exhaustive treadmill exercise and the top 20% were randomly bred to produce the HCR strain, whereas the bottom 20% produced the LCR strain. Other than the exhaustive bout of exercise for phenotyping purposes, rats were not exposed to additional exercise throughout the study.

Male LCR and HCR rats ($n = 40$) were used for the purpose of this investigation and were individually housed in a temperature-controlled environment with a 12:12-h light-dark cycle throughout their lifespan. Animals were maintained on standard laboratory chow (14 % energy

from fat; Purina Mills Prolab RMH 3000 5P00) and water ad libitum until they were weight stable. At 6 months of age, the diet for two groups (n = 10) of LCR and HCR rats was changed to high fat for 12 weeks (50 % energy from fat; Purina Mills Prolab RMH 3000 58A0) while remaining animals continued on the same chow. Because run time to exhaustion varies significantly within these strains, animals were assigned to assure similar mean run times between the dietary groups. On days when tissues were harvested, rats were fasted for 4 h to control for variability in feeding behavior, anesthetized using 0.1 ml/100g body wt of a mixture containing 90 mg/ml ketamine and 10 mg/ml xylazine, and tissues were harvested. All procedures were approved by the Animal Care and Use Committee at East Carolina University.

Oral glucose tolerance test

Following a period of acclimatization to gavage, the animals underwent an oral glucose tolerance test (OGTT) before and following the 12-wk diet treatment period. After a 10-h overnight fast, blood was collected from the tail vein to determine baseline serum glucose and insulin values. After the baseline draw, animals received a glucose load (2 g glucose/kg body wt) followed by additional blood draws at 30, 60, and 120 min. Glucose levels were determined at the moment of collection using the glucose oxidase method (OneTouch Ultra glucose analyzer; Lifescan, Milpitas, CA). Remaining blood samples were left to clot for 30 min on ice, followed by centrifuging at 4°C and 2500g for 20min to separate serum. Serum was stored at -80°C until insulin levels could be determined via a rat/mouse ELISA kit (Linco Research, St. Charles, MO). Results from these analyses were used to determine glucose tolerance by area under the glucose and insulin curves. In addition, homeostasis model assessment (HOMA) insulin resistance values were determined as calculated by fasting insulin ($\mu\text{U/ml}$) $\times$ fasting glucose (mM)/22.5.

Preparation of skeletal muscle homogenates

With blood flow intact, mixed gastrocnemius (MG) was dissected from anesthetized rats, snap frozen within seconds in liquid nitrogen and stored at -80° C in gasket sealed cryogenic vials. On the day of analysis, muscles were pulverized by a liquid nitrogen cooled stainless steel mortar and pestle. Frozen muscle samples (50–80 g) were subsequently homogenized in ice-cold lysis buffer [50mM HEPES, 50mM Na⁺ pyrophosphate, 100mM Na⁺ fluoride, 10mM EDTA, 10mM Na⁺ orthovanadate, 1% Triton X-100, and protease and phosphatase (1 and 2) inhibitor cocktails (Sigma, St. Louis, MO)]. Homogenates were sonicated for 10 sec then rotated for 2h at 4°C. After centrifugation for 25 min at 15 000 g, supernatants were extracted and protein content was detected using a BCA protein assay (Pierce, Rockford, IL) and individual homogenate volumes were separated into 50 μg (for Western) and 200 μg (for immunoprecipitation) of protein before being frozen in liquid nitrogen and stored at -80°C until used for immunoblotting.

Western blotting

For IRS-1, homogenates were subjected to 10 μl IRS-1 monoclonal antibody (Santa Cruz Biotech, Santa Cruz, CA) overnight then coupled to protein A sepharose beads and rotated for 2 hours (Amersham Biosciences, Uppsala Sweden) and eluted with a 1:10 solution of Bond Breaker TCEP (Pierce, Rockford, IL) and Laemmli Sample Buffer (Biorad, Hercules, CA). Samples were separated by SDS-PAGE using 7.5% or 10% Tris-HCl gels (Biorad) and then transferred to PVDF membranes for probing by appropriate antibodies. Membranes were probed for IRS1 and Phospho-IRS1 Ser³⁰⁷ (Millipore, Billerica, MA) and I κ B α (Cell Signaling Technologies, Beverly, MA). Following incubation with primary antibodies, blots were incubated with appropriate horseradish peroxidase-conjugated secondary antibodies. Horseradish peroxidase activity was assessed with ECL solution (Thermo Scientific, Rockford, IL), and exposed to film. The image was scanned and band densitometry was assessed with

Gel Pro Analyzer software (Media Cybernetics, Silver Spring, MD). Phospho-IRS1 Ser³⁰⁷ content was calculated from the density of the band of Phospho-IRS1 Ser³⁰⁷ divided by the density of the protein using the appropriate antibody. Horseradish peroxidase-conjugated anti-rabbit secondary antibodies were purchased from Cell Signaling Technologies (Beverly, MA).

Statistical analysis

All data are presented as means $\pm$ SEM. A two-way analysis of variance (group X diet treatment) was used to compare group means (SPSS, Chicago, IL). Where an interaction was observed, post hoc analysis (Bonferroni and Tukey's HSD) was used to determine the site of statistical significance. Correlation analysis was performed by the Pearson product-moment method. Significance level was established a priori at $p \leq 0.05$.

Results

Glucose tolerance

Following HFD treatment, glucose values were similar in the HCR animals maintained on chow diet. In contrast, the LCR rats demonstrated significantly higher glucose AUC following HFD when compared with the HCR counterparts ($p < 0.05$; • Fig. 1A). When compared to HCR rats on chow diet, LCR animals prior to HFD treatment demonstrated a significantly higher insulin AUC ($p < 0.05$; • Fig. 1B) suggesting poorer insulin action vs. HCR rats. The area under the curve for insulin was significantly greater ($p < 0.05$) in LCR rats following the HFD suggesting further reductions in insulin action on the diet. Interestingly, the insulin AUC following the OGTT in HCR animals was not elevated following HFD suggesting a metabolic resistance or protection from reduced insulin action typically observed following a chronic high lipid diet.

IKK β action

IKK β has been implicated in inhibiting insulin signaling in a variety of tissues. We measured I κ B α levels in MG as a surrogate for IKK β activity. I κ B α is rapidly degraded upon phosphorylation by IKK β , thus it is an inverse indicator of IKK β activity. I κ B α levels in LCR rats was approximately 13 % lower on the chow diet when compared with HCR rats and this difference increased to ~47% on the HFD ($p < 0.05$), indicating elevated IKK β activity in the LCR compared with the HCR rats (• Fig. 2A). As true for the glucose and insulin responses, I κ B α levels did not differ between diets in the HCR rats. In contrast, I κ B α levels in the LCR rats on the HFD were approximately 28% lower ($p < 0.05$) compared with LCR rats on a chow diet.

IRS-1 Serine³⁰⁷ phosphorylation

Phosphorylation of IRS-1 Ser³⁰⁷ is a known mediator of IKK β -induced insulin resistance. To test whether the observed differences in IKK β activity between groups and diets may be partly responsible for the differences in basal glucose and insulin levels, we determined IRS-1 pSer³⁰⁷ and total IRS-1 levels. IRS-1 pSer³⁰⁷ levels in the LCR rats increased by roughly 31 % on the HFD versus chow ($p < 0.01$; • Fig. 2B). However, no significant change was observed between diets in the HCR rats.

In order to better understand the relationship between IKK β activity and IRS-1 pSer³⁰⁷, we performed a correlational analysis between the two independent variables. Results of the analysis indicated a positive and marginally significant relationship between levels of I κ B α and IRS-1 pSer³⁰⁷ ($p < 0.05$, $r = -0.454$; • Fig. 3).

Discussion

The major findings of the current investigation are: 1) In both the HFD and chow diet, the LCR rats had lower levels of I κ B α compared with the HCR rats, indicative of increased IKK β activity. Moreover, I κ B α levels in the LCR rats were further reduced with HFD relative to chow-fed LCR rats and 2) IRS-1 pSer³⁰⁷ levels increased on the HFD versus chow, but only in the LCR animals. Correlation analysis revealed a negative relationship between levels of I κ B α and IRS-1 pSer³⁰⁷, which offers support for IKK β 's role as an IRS-1 Ser³⁰⁷ phosphorylating protein and a mediator of insulin resistance in skeletal muscle.

Elevated oxidative capacity, such as occurs via endurance exercise training, is believed to protect against the development of obesity and diabetes. Rats bred both for low (LCR) and high (HCR) capacity endurance running provide a genetic model with inherent differences in aerobic capacity that allows for the testing of this supposition without the confounding effects of a training stimulus. Novel results from an earlier study from our lab [16] indicated that chow-fed LCR rats were heavier, hypertriglyceridemic, less insulin sensitive, and had lower skeletal muscle oxidative capacity compared to HCR rats. Upon exposure to a HFD, LCR rats gained more weight and fat mass, and their insulin-resistant condition was exacerbated, despite consuming similar amounts of metabolizable energy as chow-fed controls. These metabolic variables remained unaltered in HCR rats. Interestingly, the HFD increased skeletal muscle oxidative capacity similarly in both strains. These results suggest LCR rats are predisposed to obesity but expansion of skeletal muscle oxidative capacity does not prevent excess weight gain or the exacerbation of insulin resistance on a HFD. Therefore, the purpose of this study was to explore alternative mechanisms to explain these initial results.

Evidence for IKK β involvement in reduced insulin signaling and resistance in skeletal muscle has gained increasing support recently. For example, *Ikk β ^{-/-}* mice have lower fasting glucose and insulin concentrations compared to *Ikk β ^{+/+}* littermates when on a high-fat diet [22], and IKK β KO mice are resistant to the insulin-resistant effects of a lipid infusion [14]. Lipid accumulation is known to play a causative role in the pathogenesis of insulin resistance [7] and activate IKK β [1] and the previous observation that LCR rats have a reduced ability to oxidize lipids compared with the HCR rats [16] prompted the investigation of IKK β activity in this study. The current investigation demonstrates that LCR rats have elevated IKK β activity compared with the HCR rats on both chow and HFD. Moreover, in the LCR strain, IKK β activity was increased in the HFD compared with the chow-fed rats.

IKK β , a serine kinase, phosphorylates the Ser³⁰⁷ residue on IRS-1 and, due to Ser³⁰⁷'s proximity to the phosphotyrosine-binding (PTB) domain in IRS-1 [15], leads to attenuation in insulin signal transduction [4,5,17,22]. In an effort to explain the contrast in glucose handling between the strains and diets, and considering the observed differences in I κ B α levels, an inverse indicator of IKK β action, we measured IRS-1 pSer³⁰⁷ in mixed gastrocnemius skeletal muscle and found elevated levels in the LCR rats on the HFD compared with all other groups. Moreover, the correlation between I κ B α and IRS-1 pSer³⁰⁷ was statistically significant, providing further support for IKK β 's role in phosphorylating IRS-1 Ser³⁰⁷. Additionally, when comparing the HOMA values (use of fasting glucose and insulin as an index of insulin resistance) determined previously [16] ($\bullet$ Fig. 4) with IRS-1 pSer³⁰⁷ levels ($\bullet$ Fig. 3), we observed the same overall trends between groups mirrored one another. In particular, the HOMA value in the LCR-HFD group was substantially higher (indicating greater insulin resistance) than the other groups, which is similar to that which we observed with IRS-1 pSer³⁰⁷ levels.

Studies into the effects of training on NF- κ B pathway intermediates have revealed that exercise results in various responses with regard to the NF- κ B pathway in muscle in a time-dependent

manner. Whereas an immediate measurement of muscle proteins following a single exercise bout reveals elevated IKK β activity and reduced I κ B α levels [9], a more prolonged period following exercise is associated with a diminution in muscle IKK β activity and an increase in I κ B α levels compared to pre-exercise values in both humans and rats [18,20]. Moreover, this same period of time that reveals a decrease in IKK β activity has been shown to have reduced IRS1-Ser³⁰⁷ phosphorylation in gastrocnemius muscle and improved whole-body insulin sensitivity in exercised rodents on a HFD [18]. In addition to a single bout of exercise, a chronic exercise stimulus has also been shown to result in improvements in insulin handling and inflammatory profile. Specifically, Sriwijitkamol et al. [21] observed that type 2 diabetics had reduced I κ B α levels compared with control subjects, and that this value directly correlated with insulin-mediated glucose disposal. Finally, after an 8-wk training stimulus, I κ B α levels increased by 50 % in vastus lateralis, indicating reduced IKK β activity. While the current study does not involve a training stimulus, it is noteworthy that the HCR rats exhibit similar characteristics in regards to IKK β activity and IRS-1 Ser³⁰⁷ phosphorylation that result from chronic training. Therefore, the development of a novel animal model established by selective breeding for high vs. low endurance capacity provides researchers with the means to address genetic factors related to aerobic capacity that protect or predispose individuals to the metabolic syndrome and could prove immensely valuable in terms of detecting potential therapeutic targets, especially for those where exercise is not an option due to mitigating circumstances as a consequence of chronic metabolic disease.

In summary, LCR rats are less insulin sensitive than HCR rats, and this difference becomes more pronounced on a HFD. Furthermore, the contrast in insulin resistance (measured by HOMA) followed a similar trend to levels of Ser³⁰⁷ phosphorylation in MG, with the HFD-fed LCR rats having both the highest HOMA values and IRS-1 pSer³⁰⁷ levels. Interestingly, the HFD-fed LCR rats also had the highest IKK β activity. I κ B α and IRS-1 pSer³⁰⁷ levels were significantly correlated, offering further support of IKK β 's role in phosphorylating IRS-1 on serine³⁰⁷ in skeletal muscle. Overall, whereas a high aerobic capacity appears to offer protection from the deleterious effects of high-fat feeding, these results suggest that a diminished aerobic capacity and a high-fat diet, a scenario that describes a typical Western lifestyle, combine to result in attenuation of insulin signaling and exacerbation of insulin resistance.

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Fig. 1.

Area under the curve (AUC) for glucose (A) and insulin (B) levels taken at 0, 30, 60, and 120 min from an oral glucose tolerance test (2 g glucose/kg body wt) performed on HCR and LCR rats on chow and HFD for 12 wk. $p < 0.05$ for LCR vs. HCR within the same diet (*) and chow vs. HFD within a given strain (#).

Fig. 2.

Skeletal muscle protein levels from mixed gastrocnemius (MG). Western blot analysis was performed from tissue homogenates for IκBα levels (A), as a surrogate for IKKβ activity, and phospho-Ser³⁰⁷ on IRS-1 (pSer³⁰⁷:total IRS-1; B). $p < 0.05$ for LCR vs. HCR within the same diet (*) and chow vs. HFD within a given strain (#).

Fig. 3. The relationship between IkBα and pSer³⁰⁷ (C). IkBα and IRS1-pSer³⁰⁷ levels were significantly related in WG ($r = -0.431$, $p < 0.05$).

Fig. 4.

Homeostasis model assessment (HOMA) was employed to determine insulin resistance.

Fasting glucose and insulin values were used to determine HOMA values for each rodent. $p < 0.05$ for LCR vs. HCR within the same diet (*) and chow vs. HFD within a given strain (#).