MARCELO BENEDITO DA SILVA FLORES

PAPEL DA LEPTINA E INSULINA NA VIA PI 3-QUINASE/AKT EM HIPOTÁLAMO DE RATOS SUBMETIDOS A EXERCÍCIO FÍSICO

CAMPINAS

2006

MARCELO BENEDITO DA SILVA FLORES

PAPEL DA LEPTINA E INSULINA NA VIA PI 3-QUINASE/AKT EM HIPOTÁLAMO DE RATOS SUBMETIDOS A EXERCÍCIO FÍSICO

Dissertação de Mestrado apresentada à Pós-Graduação da Faculdade de Ciências Médicas da Universidade Estadual de Campinas, para obtenção do titulo de Mestre em Clínica Médica, área de concentração em Ciências Básicas.

ORIENTADOR: *Prof. Dr. José Barreto Campello Carvalheira*

CAMPINAS

2006

Título em inglês : Exercise improves insulin and leptin sensitivity in hypothalamus of wistar rats

Keywords:
- Leptin
- Insulin
- Hypothalamus
- Rats

Área de concentração : Ciências Básicas

Titulação: Mestrado em Clinica Médica

Banca examinadora: Prof Dr José Barreto Campello Carvalheira
Profa. Dra. Elaine Beraldi Ribeiro
Prof Dr Sigisfredo Luís Brenelli

Data da defesa: 17- 02- 2006

Eliane
(Currículo Lattes)

Banca Examinadora da Dissertação de Mestrado

Orientador(a): ***Prof. Dr. José Barreto Campello Carvalheira***

Membros:

1. Prof(a). Dr(a) Eliane Beraldo Ribeiro
2. Prof(a). Dr(a). Sigisfredo Luis Brenelli
3. Prof(a). Dr(a). José Barreto Campello Carvalheira

Curso de Pós-Graduação em Clínica Médica, área de concentração Ciências Básicas da Faculdade de Ciências Médicas da Universidade Estadual de Campinas.

Data: 17/02/2006

Aos meus pais, Mª Benedita e José, Mª Apparecida e Manoel, e aos meus irmãos pelo estímulo e confiança.

AGRADECIMENTOS

Ao meu orientador "Zé Barreto", pela sua orientação competente e desafiadora, características que me levaram à autonomia nas formas de pensar e fazer ciência.

Ao "Brenelli", pelo incentivo ao meu ingresso na pós-graduação, e sua amizade.

Ao Dr. Mário e seus alunos.

Ao Dr. Lício e seus alunos.

Aos amigos que de forma especialíssima estiveram ao meu lado durante esse período:

Luis Fernando *Marília*

Cassiana ***Fabio shikasho*** ***Érika Anne***

Mirian

Marcel ***Maria Esméria***

Cafu ***Vivian***

Fernanda

Patrícia Prada *Josenilson* *Sr. Lu*

Dani e Denis

Márcio "do Biotério" ***Glaydson e Adilton***

Talita ***Rosa***

Maristela *Sr. Jósimo*

Eduardo

Juliana *Graziela*

Rodrigo

Paty Karoll *Christine*

SUMÁRIO

α-MSH	Hormônio estimulador de melanócitos
ACTH	Hormônio adenocorticotrófico
AgRP	Peptídeo relacionado ao agouti
Akt/PKB	Proteína quinase B
ATP	Trifosfato de Adenosina
GABA	Ácido gama-aminobutírico
CART	Peptídeo regulado por cocaína e anfetamina
CRH	Hormônio liberador de corticotrofina
CRF	Fator liberador de corticotrofina
DTT	Ditiotreitol
EDTA	Ácido etilenodiaminotetracético
ERK	Quinase regulada por sinal extra-celular
Gab-1	Proteína associada ao Grb2
GLUT-4	Isoforma 4 do transportador de glicose
ICV	Intracerebroventricular
IL-6	Interleucina-6
IκB	Inibidor do NFκB

IKK	Quinase do IκB
IR	Receptor de insulina
IRS-1	Substrato 1 do receptor de insulina
IRS-2	Substrato 2 do receptor de insulina
JAK	Janus quinase
JNK	Quinase da c-Jun
LH	Hipotálamo lateral
MAPK	Proteína quinase ativadora de mitose
MCH	Hormônio concentrador de melanina
NE	Norepinefrina
NPY	Neuropeptídeo Y
OBR	Receptor de leptina
OBRl	Forma longa do receptor de leptina
OBRs	Forma curta do receptor de leptina
PI3K	Fosfatidilinositol3-quinase
PKC	Proteína quinase dependente de cálcio
PMSF	Fluoreto de fenilmetil sulfona
POMC	Pro-opiomelanocortina
PTP1b	Proteína tireosina fosfatase 1b

PVN	Núcleo paraventricular
Rpm	Rotações por minuto
SDS-PAGE	Eletroforese em gel de poliacrilamida com dodecil sulfato de sódio
Shc	Substrato do receptor de insulina homólogo a Src
SHP-2	Proteína tirosina fosfatase
SOCS	Supressor da sinalização de citocinas
STAT	Transdutor de sinal e ativador de transcrição
TNFα	Fator de necrose tumoral-alfa
TRH	Hormônio liberador de tirotrofina
Tris	Tri (hidroximetil)-aminometano

RESUMO

O efeito do exercício físico prolongado de média a alta intensidade interfere, profundamente, no balanço energético de ratos. Em ratos machos, em particular, programas de exercício de moderados a muito intensos levam à redução da ingestão alimentar, o que leva ao menor acúmulo energético. Entretanto as causas exatas para os efeitos supressores do apetite, advindas do exercício não são totalmente conhecidas. Recentemente, demonstrou-se neste laboratório que a insulina e a leptina têm seus efeitos anorexígenos mediados via PI 3-quinase. Neste trabalho, foi verificado se o exercício altera a expressão e/ou atividade de proteínas envolvidas na transmissão hipotalâmica do sinal insulínico depois de estimulação com insulina e leptina. Ratos machos nadaram 6 horas por 1 dia. Foram tratados com insulina ou leptina através de infusão (icv) imediatamente após o término do exercício. A infusão de insulina ou leptina reduziu a ingestão alimentar do grupo exercitado mais acentuadamente do que o verificado no grupo sedentário. A fosforilação dos IRS–1/2, a associação da PI 3-quinase aos IRSs e a fosforilação da AKT estimulados por insulina ou leptina em hipotálamos dos ratos sedentários estavam diminuídas se comparadas aos ratos exercitados; interessantemente, a fosforilação basal em serina da AKT estava mais alta nos ratos exercitados. O presente trabalho oferece parâmetros diretos da sinalização insulínica hipotalâmica, e comprova o aumento da sensibilidade do hipotálamo para a sinalização da insulina e da leptina em ratos exercitados. Estes dados dão suporte para a hipótese de que o exercício pode ter ações supressoras do apetite mediadas pela via hipotalâmica da PI 3-quinase.

ABSTRACT

Prolonged exercise of medium to high intensity is known to profoundly affect energy balance in rats. In male rats, in particular, moderately to severely intense programs lead to a reduction in food intake that contributes to retard deposition of energy as both fat and protein constituents. However the exact causes for the appetite-supressive effects of exercise are not known. Recently we and others have demonstrated that insulin and leptin have their anorectic-induced effect mediated by the PI 3-kinase pathway. In the present study we determined whether exercise alters the expression and/or activity of proteins involved in insulin-signal transduction in hypothalamus after leptin or insulin stimulus. Male Wistar rats swam 6h/day for one day. Then, animals were treated with or without intracerebroventricular (i.c.v) insulin or leptin immediately after the exercise bout. I.c.v. insulin or leptin infusion reduced food intake in exercised rats to a greater extent than that observed in sedentary rats. Insulin or leptin-stimulated phosphorylation of IRS-1/2, the associations of PI 3-kinase to IRS-1/2 and phosphorylation of Akt in hypothalamus were decreased in sedentary rats compared to exercised rats, interestingly basal serine phosphorylation of Akt was higher in the exercised rats. The present study provides direct measurements of insulin signaling in hypothalamus, and documents increased sensitivity to insulin and leptin signaling in hypothalamus of exercised rats. These findings provide support for the hypothesis that exercise could have appetite-suppressive actions mediated by the hypothalamic PI 3-kinase pathway.

1 - INTRODUÇÃO

A obesidade é a doença nutricional mais comum do ocidente. O aumento da massa corporal está associado ao desenvolvimento de hipertensão arterial sistêmica, diabetes mellitus, colecistopatia crônica calculosa, dislipidemia, doenças cardíacas e algumas formas de câncer (GRUNDY e BARNETT, 1990). A obesidade é uma doença atualmente de caráter epidêmico e está rapidamente se tornando um grave problema de saúde pública (KOPELMAN, 2000).

Não obstante a universalização dos estudos sobre a obesidade, seu mecanismo molecular permanece amplamente desconhecido. No início de 1953, Kennedy elaborou a seguinte teoria: quando um animal se alimenta mais que o necessário, a "gordura extra", de alguma, forma sinaliza ao cérebro que o corpo está mais alimentado que o normal (KENNEDY, 1953). Com efeito, o animal passa a comer menos e gastar mais energia, o que resulta em um controle da gordura corporal preciso (variação de aproximadamente 1% ao longo de vários anos).

Dois modelos animais de síndromes de obesidade geneticamente determinada vêm sendo intensivamente investigados nos últimos 30 anos: os camundongos *ob/ob* e *db/db*. Estes camundongos têm fenótipos idênticos, pesam três vezes mais e apresentam um aumento de cinco vezes da gordura corporal comparado aos camundongos controle (mesmo quando alimentados com a mesma dieta), além de serem diabéticos.

Os primeiros estudos informativos sobre os defeitos primários nestes animais foram obtidos com experimentos em que se realizava conexão parcial dos sistemas circulatórios de camundongos através de enxertos (parabiose) (COLEMAN, 1973). A parabiose realizada entre camundongos *ob/ob* e controle magro, resulta em normalização do peso do mutante *ob/ob*. Isto sugere que o camundongo *ob/ob* é deficiente de um fator circulante que pode ser reposto pelo sangue do animal controle. Contrariamente, a parabiose entre camundongos *db/db* e controle magro não normaliza o peso corporal do mutante, sugerindo que o camundongo *db/db* apresenta uma alteração na capacidade de responder ao fator que induz à saciedade. Entretanto essas conclusões não foram muito valorizadas e a sua confirmação só foi possível após a clonagem e seqüenciamento do produto do gene *ob*, a leptina, do grego *leptos*; magro (ZHANG et al., 1994).

Transmissão do Sinal de Insulina e Leptina em Hipotálamo de Ratos: Inter-relações e Implicações Fisiopatológicas

A leptina é expressa principalmente no tecido adiposo e em menores quantidades no epitélio gástrico e placenta (MAFFEI et al., 1995; MASUZAKI et al., 1997; BADO et al., 1998). A proteína do gene *ob* presente no plasma de camundongos normais, como um monômero com peso molecular de 16 kDa, não foi detectada em plasma de camundongos *ob/ob*, e foi observada em concentrações elevadas em camundongos *db/db* (HALAAS et al., 1995). A administração de leptina a camundongos *ob/ob* resulta em diminuição da ingestão alimentar, perda de peso e redução dos níveis glicêmicos (CAMPFIELD et al., 1995), além de aumentar a atividade simpática em tecido adiposo marrom, com conseqüente aumento do gasto energético (PELLEYMOUNTER et al., 1995). Entretanto o mesmo resultado não foi observado quando este hormônio foi injetado nos animais *db/db*.

Os níveis séricos de leptina correlacionam-se de forma positiva com o índice de massa corporal na grande maioria das populações estudadas (FREDERICH et al., 1995; MAFFEI et al., 1995; CONSIDINE et al., 1996; HAVEL et al., 1998). A secreção desse hormônio diminui com o jejum prolongado e estímulo β-adrenérgico (AHIMA et al., 1996) e aumenta em resposta à administração de insulina e glicocorticóides (DE VOS et al., 1995; SALADIN et al., 1995). A leptina é secretada de forma pulsátil e inversamente relacionada à atividade do eixo ACTH-Cortisol, ou seja, ocorre diminuição da secreção de leptina ao amanhecer e aumento no final da tarde (LICINIO et al., 1997).

A leptina produzida pelo tecido adiposo informa o estado nutricional do indivíduo a centros hipotalâmicos, que regulam a ingestão alimentar e o gasto energético. Assim, a redução da quantidade de tecido adiposo leva à diminuição dos níveis circulantes de leptina, estimulando a ingestão alimentar e reduzindo o gasto energético. Contrariamente, o aumento do estoque de tecido adiposo está associado à elevação dos níveis séricos de leptina, diminuindo a ingestão alimentar e aumentando o gasto energético. Através deste mecanismo, o peso do indivíduo se mantém estável durante vários anos.

Por que, então, alguns indivíduos desenvolvem obesidade e outros não? Acredita-se que a sensibilidade à leptina seja variável e que indivíduos obesos sejam resistentes à leptina (MAFFEI et al., 1995; CONSIDINE et al., 1996; FRIEDMAN e HALAAS, 1998). Apenas uma ínfima parte da população obesa tem baixos níveis séricos de leptina e desenvolvem obesidade de forma semelhante ao camundongo *ob/ob* (MONTAGUE et al., 1997; FAROOQI et al., 2001).

Vários mecanismos podem contribuir para a resistência à leptina, como a redução do transporte de leptina através de células endoteliais e da barreira hematoencefálica impedindo a chegada da leptina no fluido intersticial cerebral. Entretanto não é claro se alterações nesse processo podem levar à obesidade, mas resultados de pesquisas indicando que humanos obesos têm menor relação entre a leptina liquórica e a plasmática, se comparados com indivíduos controle, são condizentes com essa possibilidade (CARO et al., 1996). Outra potencial causa de resistência à leptina é a redução da sensibilidade hipotalâmica a esse hormônio. Esta alteração pode ser observada em roedores (rato Zucker (DA SILVA et al., 1998) e camundongo *db/db* (LEE et al., 1996)) que apresentam mutações do receptor de leptina, assim como em ratos alimentados com dieta hiperlipídica (EL-HASCHIMI et al., 2000), demonstrando que fatores ambientais são capazes de modular a via de sinalização da leptina.

A identificação de receptores específicos para leptina em plexo coróide de ratos levou a uma melhor compreensão de como acontece a sinalização da leptina no sistema nervoso central e motivou o desenvolvimento de estudos visando o esclarecimento dos mecanismos envolvidos na gênese da resistência à leptina. O receptor de leptina (OBR) é membro da família gp130 da classe I dos receptores de citocinas (TARTAGLIA et al., 1995). É encontrado em muitos tecidos com várias formas de *splicing*, sendo as mais encontradas a forma curta (OBR_S), expressa em vários tecidos, que apresenta domínios intracelulares truncados, e a forma longa (OBR_L), que apresenta domínios intracelulares longos e é expressa principalmente no hipotálamo (núcleos paraventricular, arqueado, ventromedial e dorsomedial (MERCER et al., 1996; WOODS et al., 1996)).

O OBR_S não tem sua função bem definida, mas parece influir no transporte da leptina através da barreira hematoencefálica e talvez contribua para a depuração da leptina atuando como uma fonte de receptor solúvel.

A homologia do receptor de leptina à classe I dos receptores de citocinas forneceu informações importantes para a descoberta dos possíveis mediadores intracelulares da ação da leptina. Os receptores da classe I das citocinas agem através das famílias das proteínas JAK (*Janus Kinase*) e STAT (*Signal Transducers Activators of Transcription*) (HELDIN, 1995). Tipicamente, as proteínas JAK estão constitutivamente associadas com seqüências de aminoácidos dos receptores, e adquirem sua atividade tirosina quinase após a ligação do hormônio a seu receptor. Uma vez ativada, a proteína JAK fosforila o receptor induzindo à formação de um sítio de ligação para as proteínas STAT, as quais são ativadas após terem se associado ao receptor e serem fosforiladas pela JAK. As proteínas STAT ativadas são translocadas para o núcleo e estimulam a transcrição.

O OBR_L é capaz de estimular as proteínas STAT em resposta à sua ativação. Dois estudos (BAUMANN et al., 1996; GHILARDI et al., 1996) mostraram que a leptina ativa o STAT3 e o STAT5 em células COS transfectadas com o OBR_L, mas discordaram em relação à atividade do STAT1 e do STAT6. A proteína da família STAT mais importante para a regulação do peso corporal ainda não foi identificada. No entanto é pouco provável que tanto o STAT1 como o STAT6 estejam envolvidos de forma significativa, uma vez que a ausência de expressão desses genes em camundongos *knockout* não resultou em obesidade (DURBIN et al., 1996; MERAZ et al., 1996; SHIMODA et al., 1996; TAKEDA et al., 1996). Embora o OBR_L seja capaz de ativar as proteínas STAT3 e STAT5 em células COS, as proteínas do STAT que são realmente ativadas *in vivo* podem diferir das que são observadas nestas linhagens celulares (TARTAGLIA, 1997). Apenas a ativação do STAT3 foi detectada no hipotálamo de camundongos, após a administração exógena de leptina (VAISSE et al., 1996).

O receptor de leptina é capaz de estimular outras vias de sinalização além da JAK/STAT, tais como a via da proteína quinase ativadora de mitose (MAPK) e a via de fosfatidilinositol 3-quinase (PI 3-quinase) (HELDIN, 1995), e é possível que a capacidade

do OBR controlar o peso dependa também destas vias de sinalização (TARTAGLIA, 1997; NISWENDER et al., 2001). Além disso, a leptina leva também à fosforilação do SHP2 (CARPENTER et al., 1998), uma fosfotirosina fosfatase, que diminui o grau de fosforilação da JAK2 e conseqüentemente a ativação do STAT3. Uma outra proteína, SOCS3, quando ativada, diminui a resposta à leptina (BJORBAEK et al., 1998).

Após a ativação dos receptores de leptina no cérebro e das proteínas envolvidas na transmissão do sinal desse hormônio, respostas neuronais integradas são necessárias para modular a ingestão alimentar e o gasto energético. Alguns neurotransmissores importantes para o funcionamento dessa rede neuronal estimulam a ingestão alimentar como o neuropeptídeo Y (NPY) (STEPHENS et al., 1995) e o *Agouti related peptide* (AGRP) (SHUTTER et al., 1997), enquanto outros provocam redução da ingestão alimentar como o *cocaine-and anphetamine–regulated transcription* (CART) (KRISTENSEN et al., 1998) e o *melanocyte stimulating hormone* (α-MSH) (FAN et al., 1997). A leptina regula o balanço energético diminuindo os níveis de neuropeptídeos anabólicos NPY e AGRP e aumentando a concentração de neuropeptídeos catabólicos CART e α-MSH.

Assim como a leptina, a insulina também é considerada um hormônio que sinaliza ao hipotálamo o estoque de tecido adiposo e modula a ingestão alimentar (WOODS et al., 1979; WOODS et al., 1985). A insulina circula em níveis proporcionais ao conteúdo de tecido adiposo e atravessa a barreira hematoencefálica via um sistema de transporte saturável em níveis proporcionais aos plasmáticos (BAURA et al., 1993). Os receptores de insulina são expressos por neurônios envolvidos na ingestão alimentar (BASKIN et al., 1988; CHEUNG et al., 1997; BASKIN et al., 1999). A administração de insulina no sistema nervoso central reduz a ingestão alimentar e diminui o peso corporal, enquanto a deficiência desse hormônio causa hiperfagia (SIPOLS et al., 1995).

A correlação dos níveis séricos de insulina com o conteúdo de gordura corporal é conseqüência da resistência à insulina induzida pelo aumento da gordura corporal (SCHWARTZ et al., 1997). Assim, à medida que o peso corporal aumenta a insulina deve aumentar para compensar a resistência à insulina e manter a homeostase de glicose (POLONSKY et al., 1988; KAHN et al., 1993). A falência da célula β em alcançar este aumento adaptativo causa hiperglicemia, e provavelmente contribui para a associação entre

diabetes tipo 2 e obesidade. Acredita-se que o aumento progressivo da secreção de insulina que ocorre durante o desenvolvimento da obesidade atue como uma alça de retroalimentação limitando o acúmulo de tecido adiposo.

Várias observações indicam que a leptina desempenha uma função mais importante que a insulina no hipotálamo para controle da homeostase energética. Por exemplo, a deficiência de leptina causa obesidade grave, com hiperfagia, que persiste apesar dos altos níveis circulantes de insulina. Em contraste, a deficiência de insulina não causa obesidade. Entretanto este tipo de comparação é complicado pelo papel da insulina em promover a síntese de leptina e o armazenamento de tecido adiposo. Uma vez que o armazenamento de tecido adiposo requer insulina, não pode ocorrer ganho de peso quando a deficiência de insulina está presente, mesmo com um consumo de grande quantidade de alimento. Outro exemplo interessante é o diabetes descompensado, onde a ingestão alimentar aumenta marcadamente (LEEDOM e MEEHAN, 1989), mas a quantidade de gordura corporal está baixa, bem como os níveis plasmáticos de leptina (HAVEL et al., 1998; HATHOUT et al., 1999). Como tanto os níveis séricos de insulina como o de leptina estão baixos nesta situação, a "hiperfagia diabética" poderia ser resultado da diminuição da sinalização da insulina, da leptina ou de ambas. Um estudo recente esclareceu este assunto mostrando que a reposição isolada de leptina (mas não a de insulina) é capaz de prevenir a "hiperfagia diabética", indicando que a leptina realmente é mais potente que a insulina no controle central da homeostase energética. Apesar disso, recentemente, foi descrito que a inativação do receptor de insulina apenas no sistema nervoso central de camundongos resulta em aumento da ingestão alimentar e obesidade leve, aumento dos níveis plasmáticos de leptina, aumento da secreção de insulina, bem como resistência à insulina leve, demonstrando a necessidade da integridade da via de sinalização da insulina cerebral para o controle do peso corporal (BRUNING et al., 2000).

A sinalização intracelular da insulina em tecidos insulino-sensíveis inicia-se com a ligação do hormônio a um receptor específico de membrana, uma proteína heterotetramérica com atividade quinase, composta por duas subunidades α e duas subunidades β. A ligação da insulina à subunidade α estimula a autofosforilação da região intracelular da subunidade β do receptor.

Uma vez ativado, o receptor de insulina fosforila vários substratos protéicos em tirosina incluindo membros da família dos substratos dos receptores de insulina (IRS-1/2/3/4), Shc, Gab-1 e Cbl (Figura 1). A fosforilação em tirosina das proteínas IRS cria sítios de reconhecimento para moléculas contendo domínios com homologia à Src 2 (SH2). Dentre estas, destaca-se a PI 3-quinase. As funções fisiológicas do IRS-1/2 foram recentemente estabelecidas através da produção de camundongos sem os genes que codificam o IRS-1 ou IRS-2 (*knockout* de IRS-1 e IRS-2). O camundongo que não expressa IRS-1 apresenta resistência à insulina e retardo de crescimento, mas não é diabético (ARAKI et al., 1994). Foi demonstrado que o IRS-2 poderia compensar parcialmente a ausência de IRS-1, o que explicaria o fenótipo de resistência à insulina sem hiperglicemia do camundongo *knockout* de IRS-1. O camundongo que não expressa o IRS-2 foi posteriormente gerado (WITHERS et al., 1998) e mostrou um fenótipo diferente do camundongo sem IRS-1: hiperglicemia acentuada devido a diversas anormalidades na ação da insulina nos tecidos periféricos associada à falência da atividade secretória das células β. Esta última alteração é, provavelmente, conseqüência da redução significativa da massa de células β pancreáticas.

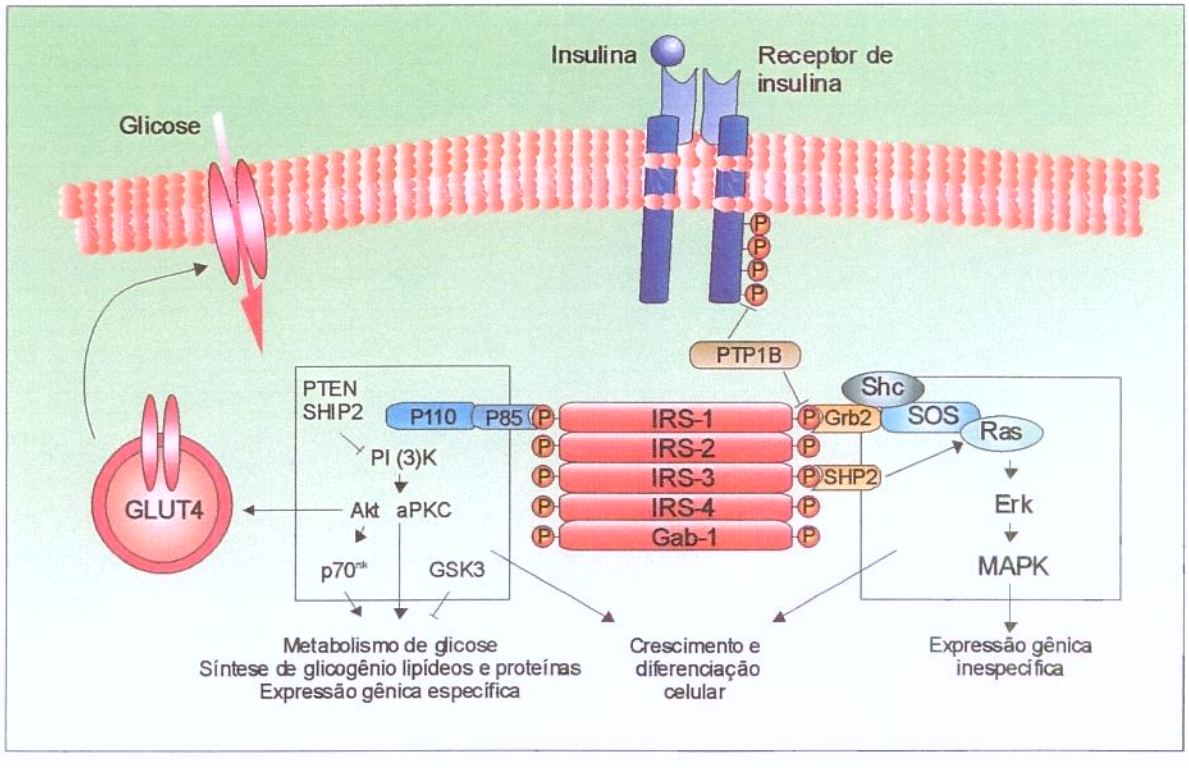


Figura 1 -Vias de sinalização da insulina.

A fosforilação das proteínas IRSs cria sítios de ligação para a PI 3-quinase, promovendo a sua ativação. Atualmente, a PI 3-quinase é a única molécula intracelular inequivocamente considerada essencial para o transporte de glicose (CZECH e CORVERA, 1999). As proteínas alvo conhecidas dessa enzima são a Akt e as isoformas atípicas da PKC (ζ e λ), porém a função dessas proteínas no transporte de glicose ainda não está bem estabelecida (KOHN et al., 1996; BANDYOPADHYAY et al., 1997; KITAMURA et al., 1998; KOTANI et al., 1998; KIM et al., 1999).

Exercício e Sistema Nervoso Central

A importância do exercício físico no sucesso de programas para perda de peso é reconhecida há vários anos (PRONK et al., 1994; MILLER et al., 1997). O exercício físico aumenta a perda de peso em curto prazo e, quando combinado à dieta, é um dos melhores preditores de perda de peso em longo prazo (PRONK et al., 1994). Entretanto o efeito do exercício nas vias neurais para controle da ingestão alimentar e gasto energético não é claro. Atualmente, existem poucos estudos que se voltam para a explicação da relação entre o exercício e as vias hipotalâmicas que regulam a homeostasia energética (RIVEST e RICHARD, 1990; BOVETTO e RICHARD, 1995; LEVIN et al., 2003; LEVIN e DUNN-MEYNELL, 2004). Por outro lado, estudos demonstram os efeitos do exercício sobre outras funções cerebrais que também usam neurotransmissores e fatores tróficos para o controle da homeostasia energética. Estes incluem fator liberador de corticotropina CRF (TIMOFEEVA et al., 2003), norepinefrina (NE), serotonina (5HT), GABA (DISHMAN, 1997), e fator neurotrófico derivado do cérebro (BDNF) (NEEPER et al., 1996), 1996). Agindo no hipotálamo, NE (LEIBOWITZ et al., 1984) e GABA (KELLY et al., 1977) têm efeitos anabólicos, enquanto CRF, 5HT (WALDBILLIG et al., 1981), e BNDF (LYONS et al., 1999) têm efeitos catabólicos.

Em trabalho recente (BI et al., 2005) verificaram que animais obesos submetidos a sessões aeróbicas de exercício tiveram redução significativa da adiposidade e a recuperação do peso foi retardada. Apesar de terem sido verificadas concentrações plasmáticas reduzidas de leptina, não se observou aumento da ingestão alimentar como forma compensatória para a perda de peso. Este efeito foi secundário à ação direta do exercício nos núcleos dorsomediais do hipotálamo, regulando a expressão dos neuropeptídeos CRF e NPY. Além disso, foi demonstrado que o exercício reduz a

expressão de NPY em hipotálamo de ratos diabéticos sugerindo que o exercício seja capaz de modular a ingestão alimentar e o gasto energético através circuitos neuronais (SHIN et al., 2003).

O que se pode deduzir dos experimentos com exercício em roedores que possam ser generalizados em humanos? Talvez o mais importante seja a identificação de fatores que "dizem" ao cérebro que o corpo está se exercitando. De fato, o exercício gera um número de sinais metabólicos, hormonais e neuronais que chegam até o cérebro. Dentre estes fatores, a interleucina-6 (IL- 6) é particularmente interessante pelo fato de ser liberada pelo músculo em contração e pode, juntamente à sinalização da melanocortina, aumentar a expressão de CRF no núcleo paraventricular (DISHMAN 1997) e também, possivelmente, no núcleo dorsomedial. Os estudos conduzidos por Bi *et al* apontam para um novo estado de algumas proteínas hipotalâmicas responsáveis pela homeostasia energética, que após interagirem com moléculas liberadas pelo músculo em contração, respondem por novos padrões energéticos nos ratos exercitados, como a diminuição da ingestão alimentar e o menor acúmulo adipocitário.

Exercício e Transmissão do Sinal de Insulina

Em diversas condições fisiológicas, o transporte de glicose através da membrana celular é um fator limitante na utilização de glicose pelo músculo esquelético (KUBO e FOLEY, 1986; CLINE et al., 1999). A insulina e o exercício físico são os estimuladores fisiologicos mais relevantes do transporte de glicose no músculo esquelético (HAYASHI et al., 1997; GOODYEAR e KAHN, 1998). Embora agudamente o exercício não seja capaz de aumentar a fosforilação em tirosina do IR e nem de aumentar a fosforilação em tirosina do IRS-1, estimulada por insulina (HENRIKSEN, 2002), observa-se que o exercício potencializa o efeito da insulina na fosforilação do IRS-2 com conseqüente aumento da atividade da PI(3)K (HOWLETT et al., 2002). Além disso, ocorre também uma maior fosforilação em serina da Akt, proteína fundamental para iniciar a translocação do GLUT4 para a membrana citoplasmática (WOJTASZEWSKI et al., 1999). Resultados publicados por nosso laboratório mostraram que o exercício de *endurance* melhora a sensibilidade à insulina, aumentando a fosforilação do IRS-1 e IRS-2, bem como a associação dessas proteínas com a PI(3)K em animais estimulados com insulina quando comparados aos animais controle (LUCIANO et al., 2002).

O GLUT4 é o maior transportador de glicose expresso no músculo esquelético, e a sua translocação do meio intracelular até a membrana plasmática e túbulos T constitui-se no principal mecanismo através do qual insulina e exercício efetuam o transporte de glicose no músculo esquelético (HAYASHI et al., 1997; GOODYEAR e KAHN, 1998). A atividade contrátil do músculo pode estimular a translocação do GLUT4 na ausência de insulina (HAYASHI et al., 1997; GOODYEAR e KAHN, 1998), e alguns estudos sugerem que existem dois diferentes *"pools"* intracelulares de GLUT4, um estimulado por insulina e um estimulado pelo exercício (DOUEN et al., 1990; CODERRE et al., 1995). Portanto os efeitos da insulina e da contração muscular são aditivos, sugerindo que a insulina e o exercício ativam os transportadores de glicose por diferentes mecanismos.

O estilo de vida sedentário é um fator que contribui para o desenvolvimento ou aumento da resistência à insulina (BASSUK e MANSON, 2005). Estudos demonstraram que a sensibilidade à insulina pode aumentar com exercícios físicos, independentemente da redução do peso e de mudanças na composição corporal (O'DONOVAN et al., 2005), e que o principal efeito do exercício pode ser o aumento da expressão de elementos intracelulares da via de sinalização da insulina, em particular dos transportadores de glicose (GLUT-4) na musculatura esquelética (TERAN-GARCIA et al., 2005). Interessantemente em paciente com diabetes do tipo 2, o transporte de glicose estimulado pela contração muscular se mantém sem alterações (KENNEDY et al., 1999).

Estudos experimentais demonstraram que o exercício físico em ratos diabéticos foi capaz de aumentar a fosforilação do receptor de insulina e da Akt, melhorando conseqüentemente a captação de glicose (HEVENER et al., 2000). Todos esses dados confirmam a importância da atividade física para a melhora da captação da glicose mediada por insulina.

O presente estudo avaliará a hipótese de que, além dos efeitos diretos do exercício nas vias de sinalização no músculo, o exercício também modula as vias de sinalização da insulina e leptina no sistema nervoso central propiciando uma integração mais ampla do metabolismo energético corporal.

2 - OBJETIVOS

Caracterizar os efeitos da Leptina na via JAK/STAT e PI 3-quinase/Akt no hipotálamo de ratos Wistar submetidos a exercício físico.

Caracterizar os efeitos da Insulina na via PI 3-quinase/Akt no hipotálamo de ratos Wistar submetidos a exercício físico.

Avaliar a influência da Interleucina- 6 sobre a sinalização hipotalâmica da Leptina e Insulina de ratos Wistar submetidos a exercício físico.

3 - CAPÍTULO

EXERCISE IMPROVES INSULIN AND LEPTIN SENSITIVITY IN HYPOTHALAMUS OF WISTAR RATS

Marcelo B. S. Flores, Maria Fernanda A. Fernandes, Eduardo R. Ropelle, Marcel C. Faria, Mirian Ueno, Licio A. Velloso, Mario J. A. Saad, José B. C. Carvalheira.

Department of internal medicine, FCM, State University of Campinas (UNICAMP), Campinas, SP, Brazil.

Short running title: Leptin/insulin signaling in exercised rats

Please address correspondence to:

José B. C. Carvalheira, MD.
Department of internal medicine
FCM - State University of Campinas (UNICAMP)
13081-970 - Campinas, SP, Brazil.
Fax: + 55 19 3788-8950
E-mail: carvalheirajbc@uol.com.br

ABSTRACT

Prolonged exercise of medium to high intensity is known to promote a substantial effect on the energy balance of rats. In male rats moderately to severely intense programs lead to a reduction in food intake. However, the exact causes for the appetite-supressive effects of exercise are not known. Here we show that i.c.v. insulin, or leptin infusion, reduced food intake in exercised rats to a greater extent than that observed in control animals. Exercise was associated with a markedly increased phosphorylation/activity of several proteins involved in leptin and insulin signal transduction in the hypothalamus. The regulatory role of IL-6 in mediating the increase in leptin and insulin sensitivity in hypothalamus was also investigated. Treatment with insulin or leptin markedly reduced food intake in exercised rats that were pre-treated with vehicle, although no increase in sensitivity to leptin- and insulin-induced anorexia after pretreatment with anti-IL-6 antibody was detected. The present study provides direct measurements of leptin and insulin signaling in the hypothalamus and documents increased sensitivity to these hormones in the hypothalamus of exercised rats in an IL-6-dependent manner. These findings provide support for the hypothesis that the appetite-suppressive actions of exercise may be mediated by the hypothalamus.

Prolonged exercise of medium to high intensity is known to profoundly affect energy balance (1-3). Studies of individuals who have maintained significant weight loss for more than a year have demonstrated that dieters who achieve long-term success are often those who engage in regular and extensive exercise programs (4). Whilst the energy expenditure aspects of such exercise may contribute to the effects of weight maintenance, it has been suggested that even acute exercise may also contribute to the energy balance by altering appetite and reducing food intake (4). The mechanisms underlying the effects of exercise on food intake have not yet been identified, however.

The circulating peptide, leptin, is secreted predominantly by white adipose tissue and provides feedback information on the extend of the body's fat stores to hypothalamic leptin receptors (OBR) that coordinate food intake and body weight homeostasis (5; 6). Wild-type OBR possess a number of signaling capabilities; these include activation of the Janus kinase-signal transducer and activator of transcription (JAK-STAT) (7-11) and mitogen-activated protein (MAP) kinase pathways and stimulation of tyrosine phosphorylation of the insulin receptor substrates (IRS-1 and IRS-2), phosphatidylinositol 3-kinase (PI 3-kinase) (10-13).

Insulin acts at the same hypothalamic areas as leptin to suppress feeding (6; 14). The insulin receptor (IR) is a protein tyrosine kinase that is activated by insulin binding, undergoing rapid autophosphorylation and phosphorylating intracellular protein substrates, including insulin receptor substrates (IRS-1 and IRS-2) (15; 16). Following tyrosine phosphorylation, the IRSs act as docking proteins for several SH2 domain-containing proteins, including PI 3-kinase, Grb2, SHP2, Nck and Fyn (17-21). PI 3-kinase activates two kinases: PDK1, which phosphorylates Akt on Thr-308, and a putative PDK2, which phosphorylates Akt on Ser-473, leading to an increase in Akt kinase activity (22).

The level of circulating interleukin-6 (IL-6) increases dramatically in response to exercise (23), with IL-6 being produced by working muscle (24; 25) and adipose tissue (26-28). IL-6 seems to have several important roles in metabolism, including induction of lipolysis (26; 29) and enhancement of insulin sensitivity when injected into IL-6-deficient mice (30). Furthermore, it appears that centrally acting IL-6 plays a role in the regulation of appetite, energy expenditure and body composition (30). Intracellular interactions between

different signaling systems may enhance or counterregulate hormone actions. Thus, it is possible that the effects of acute exercise on central insulin and leptin sensitivity may be dependent on IL-6. The status of leptin and insulin signaling in hypothalamus has not previously been assessed in rats after acute exercise. We, therefore, examined hypothalamic JAK-STAT and IRSs-PI 3-kinase signaling pathway as well as the role of IL-6 in insulin and leptin signaling in rats after acute exercise.

RESEARCH DESIGN AND METHODS

Materials

The reagents for sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblotting were from Bio-Rad. Tris[hydroxymethyl]amino-methane (Tris), aprotinin, ATP, dithiothreitol, phenylmethylsulfonyl fluoride, Triton X-100, Tween 20, glycerol, and bovine serum albumin (fraction V) were from Sigma Chemical Co. (St. Louis, MO). Protein A-Sepharose 6 MB, ^{125}I-protein A and nitrocellulose paper (Hybond ECL, 0.45 μm) were from Amersham Pharmacia Biotech United Kingdom Ltd. (Buckinghamshire, United Kingdom). Sodium amobarbital (Amytal) and human recombinant insulin (Humulin R) were from Eli Lilly Co. (Indianapolis, IN). Leptin was from Calbiochem (San Diego, CA). IL-6 was from Santa Cruz Biotechnology (Santa Cruz, CA). Ketamine hydrochloride was from Cristália (Itapira SP, Brazil). Antibodies to IR, IRS-1, IRS-2, Akt, JAK2, Ob-R, SOCS3, PTP1b and STAT3 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The Akt phosphoserine 473-specific antibody and the STAT3 phosphotyrosine 705-specific antibody were from New England Biolabs (Beverly, MA), and the antibody to the p85 subunit of PI 3-kinase was from Upstate Biotechnology (Lake Placid, NY). Routine reagents were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise specified.

Animals and Surgical Procedure

Male Wistar rats (60 days old/ 250-300g) from the University of Campinas Central Animal Breeding Center were used in the experiments. All experiments involving animals were in accordance with the guidelines of the Brazilian College for Animal Experimentation (COBEA) and were approved by the ethics committee at the University of Campinas. Rats were maintained on a 12-h light-dark cycle and were provided free access to water and standard rodent chow before the exercise; they were randomly assigned to one of two groups: 6-hour-exercised, or control. After an overnight fast, the rats were anesthetized with ketamine hydrochloride plus diazepan and positioned on a Stoelting stereotaxic apparatus. The implantation of an intracerebroventricular (i.c.v.) catheter into the third ventricle was performed as previously described (10). Cannula placement was

confirmed by a positive drinking response after administration of angiotensin II (40 ng/2 μL), and animals that did not drink 5 mL of water within 15 minutes after treatment were not included in the experiment.

Exercise Protocol

Rats were acclimated to swimming for 10 min per day for 2 days. The swimming protocol was performed as previously described (31). The rats swam in groups of three, in plastic barrels of 45 cm in diameter, filled to a depth of 50 cm, the water temperature was maintained at 34-35°C. Animals performed two 3-h exercise bouts, separated by one 45-min rest period.

Treatments and Measurement of Food Intake

After the last bout of exercise, animals were i.c.v. injected (2-μL bolus injection) with either vehicle, insulin (3.6 μg/μL; human insulin from E. Lilly), leptin (5 μg/μL; rat leptin from NIH), anti-IL-6 (2-μg/μL; rabbit interleukin-6 from Santa Cruz) plus leptin or insulin or vehicle (2-μL in the control animals). Thereafter, standard chow was given, and food intake was determined by measuring the difference between the weight of chow given and the weight of chow at the end of a 12-hour period. In preliminary experiments, we determined plasma glucose and serum insulin levels in animals that received i.c.v. insulin infusion. Insulinemia and plasma glucose were not altered by third ventricle insulin or saline microinjection.

Western Blot Analysis

Immediately after the last exercise bout, animals were treated with vehicle, insulin, or leptin, according to the protocols described in the preceding section, they were then decapitated and the hypothalami were removed at the time-points indicated, minced coarsely, and homogenized immediately in the solubilization buffer containing 100 mM Tris (pH 7.6), 1% Triton X-100, 150 mM NaCl, 0.1 mg aprotinin, 35 mg phenylmethylsulfonyl fluoride/mL, 10 mM Na_3VO_4, 100 mM NaF, 10 mM $Na_4P_2O_7$, and 4 mM EDTA, using a polytron PTA 20S generator (Model PT 10/35, Brinkmann Instruments, Westbury, NY) operated at maximum speed for 30 seconds and clarified by

centrifugation. Equal amounts of protein were used for immunoprecipitation followed by Western blot analysis with the indicated antibodies and ^{125}I-Protein A. ^{125}I-Protein A bound to the antipeptide antibodies was detected by autoradiography using preflashed Kodak XAR film (Eastman Kodak Co., Rochester, NY) with Cronex Lightning Plus intensifying screens (DuPont, Wilmington, DE) at –80 °C for 12 to 48 hours. Band intensities were quantitated by optical densitometry (Scion Image software, ScionCorp, Frederick, MD) of the developed autoradiographs.

PI 3-Kinase Assay

Aliquots of supernatants containing equal amounts of protein were incubated overnight at 4 °C using antibodies against IRS-1 or IRS-2, and the immunocomplexes were precipitated with a 50% solution of protein A-Sepharose 6MB. In vitro PI 3-kinase assays were performed as described (17). The ^{32}P-labeled 3-P-phosphatidylinositol was quantitated using Scion Image Software.

Statistical Analysis

Where appropriate, the results are expressed as the mean ± SEM accompanied by the indicated number of rats used in experiments. Comparisons among groups were made using parametric two-way ANOVA; where F ratios were significant, further comparisons were made using the Bonferroni test.

RESULTS

Physiological parameters measured in basal conditions after 6-h exercise. The plasma glucose level was lower in the exercised group compared to the control group (61.8 *vs* 80.2 mg/dl, Fig. 1a) and the insulin levels were also lower (0.2 *vs* 3.3 nmol/ml, Fig. 1b). Exercise did not, however, reduce plasma leptin (3.7 *vs* 4.2 ng/ml, Fig. 1c).

Intracerebroventricular leptin reduces food intake and activates the hypothalamic JAK-STAT pathway in exercised rats to a greater extent than in control animals. The effect of leptin, or its vehicle, on the control of food intake was studied by measuring the total food intake for 12h after a session of exercise and a single i.c.v. injection of leptin or its vehicle. Leptin induced reductions in the 12-h food intake in both control and exercised rats. In the exercised rats, leptin reduced food intake by about 37.1%, while in the control group it induced reductions of about 15.4%, indicating that leptin was much more effective in exercised rats (Fig. 2a).

To determine the effects of exercise upon the early steps of the leptin signaling pathway, the OBR and JAK2 tyrosine phosphorylation was assessed in the hypothalamus of exercised and control rats. Immunoprecipitation and western blotting of hypothalamic extracts were performed using the anti-OBR, anti-JAK2 and anti-phosphotyrosine antibodies. Leptin induced increases in OBR and JAK2 tyrosine phosphorylation levels in hypothalamus from both control and exercised rats. In the exercised animals, leptin increased OBR and JAK2 tyrosine phosphorylation by 6.0- and 4.7-fold respectively, compared with 2.1- and 2.7-fold increases in the hypothalami from control rats, representing increases in OBR tyrosine phosphorylation of 4.7- and 2.2-fold respectively (Fig. 2b, c-*upper panels*). The same membranes, used to detect tyrosine phosphorylation of OBR and JAK2, were reblotted with OBR and JAK2 antibodies and, as expected, there were no changes in OBR and JAK2 protein expression (Fig. 2b, c-*lower panels*). Hypothalamic extracts from exercised and control rats that were stimulated with leptin were lysed and the proteins separated by SDS-PAGE gel and blotted with pSTAT3 antibodies. In the hypothalami from exercised animals, leptin increased STAT3 tyrosine phosphorylation by 5.3-fold, compared with 2.4-fold increases in the hypothalami from control rats, representing increases in STAT3 tyrosine phosphorylation of 3.2-fold

(Fig. 2d *upper panel*). No changes were observed in STAT3 protein expression (Fig. 2d *lower panel*).

Intracerebroventricular leptin activates hypothalamic the IRSs-PI 3-kinase pathway in exercised rats to a greater extent than in control animals. Immunoprecipitation and Western blotting of hypothalamic extracts were performed using the anti-IRS-1 and anti-IRS-2 and anti-phosphotyrosine antibodies. Leptin induced increases in IRS1/2 tyrosine phosphorylation levels in hypothalamus from both control and exercised rats. In the exercised animals, leptin increased IRS-1 and IRS-2 tyrosine phosphorylation by 5.8- and 5.6-fold respectively, compared with 3.4- and 3.8-fold increases in the hypothalami from control rats, representing increases in IRS-1 and IRS-2 tyrosine phosphorylation of 2.0- and 1.6-fold respectively (Fig. 3a, c-*upper panels*).

The same membranes, used to detect tyrosine phosphorylation of IRS-1 and IRS-2, were reblotted with antibodies against the p85 subunit of PI 3-kinase. The PI 3-kinase association with IRS-1 and IRS-2 paralleled the changes in the phosphorylation of these proteins (Fig. 3a, c–*middle panels*). There were no changes in IRS-1 and IRS-2 proteins expressions (Fig. 3a, c–*lower panels*). To determine whether there was PI 3-kinase activity in IRS-1 and IRS-2 immunoprecipitates, hypothalami were prepared and immunoprecipitated with anti-IRS-1 or anti-IRS-2 antibodies from both control and exercised rats. After a treatment with leptin, there was an increase in PI 3-kinase activity associated with IRS-1 and IRS-2. In the exercised animals, leptin increased PI 3-kinase activity associated with IRS-1 and IRS-2 by 5.5- and 5.2-fold respectively, compared with 2.4- and 3.8-fold increases in the hypothalami from control rats, representing increases in PI 3-kinase activity associated with IRS-1 and IRS-2 of 2.0- and 1.6- fold, respectively (Fig. 3b, d).

Intracerebroventricular insulin reduces food intake and activates the hypothalamic IR, IRS-1/2-PI 3-kinase pathway in exercised rats to a greater extent than in control animals. The effect of insulin or its vehicle on the control of food intake was studied by measuring the total food intake for 12-h after a session of exercise and a single i.c.v. injection of insulin or its vehicle. Insulin induced reductions in the 12-h food intake in both control and exercised rats. In the exercised animals, insulin reduced food intake by

about 41.6%, while in the control group it induced reductions of about 27.8%, indicating that leptin was much more effective in exercised rats (Fig. 4a).

To determine the effects of exercise upon the early steps of the insulin signaling pathway, the IR, IRS-1 and IRS-2 tyrosine phosphorylation was assessed in the hypothalamus of trained and control rats. Immunoprecipitation and western blotting of hypothalamic extracts were done using the anti-IR, anti-IRS-1, anti-IRS-2 and anti-phosphotyrosine antibodies. Insulin induced increases in IR, IRS-1 and IRS-2 tyrosine phosphorylation levels in the hypothalamus of both control and exercised rats. In the exercised animals, insulin increased IR, IRS-1 and IRS-2 tyrosine phosphorylation by 6.0-, 5.5- and 5.8-fold respectively, compared with 2.7-, 2.3- and 2.5-fold increases in the hypothalami from control rats, representing increases in IR, IRS1/2 tyrosine phosphorylation of 3.0-, 3.6- and 3.2-fold, respectively (Fig. 4b, c, d-*upper panels*).

The same membranes, used to detect tyrosine phosphorylation of IRS-1/2, were reblotted with antibodies against the p85 subunit of PI 3-kinase. The PI 3-kinase association to IRS-1 and IRS-2 paralleled the changes in phosphorylation of these proteins (Fig. 4c, d-*middle panels*).

To determine whether there was PI 3-kinase activity in the IRS-1/2 immunoprecipitates, hypothalami from control and exercised rats treated with insulin, after a session of exercise, were prepared and immunoprecipitated with anti-IRS-1 or anti-IRS-2 antibodies. After treatment with insulin, there was an increase in PI 3-kinase activity associated with IRS-1 and IRS-2. In the exercised animals, insulin increased PI 3-kinase activity associated with IRS-1 and IRS-2 by 5.8- and 6.0-fold respectively, compared with 2.5- and 2.6-fold increases in the hypothalami from control rats, representing increases in PI 3-kinase activity associated with IRS-1 and IRS-2 of 2.0- and 1.6-fold, respectively (Fig. 5a, b).

Hypothalamic extracts from exercised and control rats that were stimulated with insulin were lysed and the proteins separated by SDS-PAGE on gel and blotted with pAkt antibodies. In the hypothalami from exercised animals, insulin increased Akt serine phosphorylation by 5.7-fold, compared with 2.8-fold increases in the hypothalami from

control rats, representing increases in Akt serine phosphorylation of 2.7-fold (Fig. 5c *upper panel*). No changes were observed in Akt protein expression (Fig. 5c *lower panel*).

Role of IL-6 in anorectic response to leptin and insulin. IL-6 expression was detected in control animals, however a significant increase was observed in exercised animals (Fig. 6a). We tested whether the inhibitory effects of leptin and insulin on food intake depend on IL-6 by i.c.v. infusion of anti-IL-6 into exercised rats. Treatment with leptin or insulin markedly reduced 12 h-food intake in exercised rats pre-treated with vehicle, although no exercise-induced leptin or insulin sensitivity was detected after pretreatment with anti-IL-6, respectively (Fig. 6b, c). Consistent with the increase in leptin sensitivity, JAK2 and STAT3 phosphorylation were induced by exercise and reversed by anti-IL-6 in a similar fashion to the food intake (Fig 6d). As shown in Figure 6e, insulin induced a significant increase in IR and Akt phosphorylation in the hypothalamus of exercised rats pretreated with vehicle. In animals pretreated with anti-IL-6, the effect of exercise in insulin signaling was reversed.

DISCUSSION

Exercise training has multiple effects on metabolism and gene expression (31). However, little is known about the mechanisms by which exercise leads to reduced appetite. Here we provide evidence for a molecular mechanism to account for increased insulin and leptin action in hypothalamus after exercise. I.c.v. insulin or leptin infusion reduced food intake in exercised rats to a greater extent than that observed in control animals. Exercise was associated with a marked increase in the phosphorylation/activity of several proteins involved in leptin and insulin signal transduction in hypothalamus. In addition, we investigated the regulatory role of IL-6 in mediating the increase in leptin and insulin sensitivity in hypothalamus. Treatment with insulin or leptin markedly reduced food intake in exercised rats that were pre-treated with vehicle, although no increase in sensitivity to leptin- and insulin anorexia was detected after pretreatment with anti-IL-6 antibody. Increased leptin and insulin action in the brain may, thus, contribute to the modulation of energy homeostasis in exercised rats.

Our data demonstrate that after exercise there were no changes in the expression of hypothalamic proteins involved in leptin and insulin signal transduction, however the phosphorylation status of these proteins was deeply modified. Exercise led to an increase in leptin- and insulin-stimulated OBR/JAK2 and IR tyrosine phosphorylation, respectively. The next step in leptin and insulin signaling may involve the tyrosine phosphorylation of IRS-1 and IRS-2. As shown above, the amounts of IRS-1 and IRS-2 were unchanged in the hypothalamus of exercised rats. In contrast, the phosphorylation of IRS-1 and IRS-2 after stimulation with leptin or insulin increased in those rats, compared with control animals. As IRS-1 and IRS-2 are the main molecules linking leptin and insulin signaling to PI 3-kinase activity, we examined the leptin and insulin induced association of IRS-1 and IRS-2 with the p85 subunit of PI 3-kinase and found it to be increased in the hypothalamus of exercised rats. After IRS-1 or IRS-2–PI 3-kinase association, PI 3-kinase is activated and may, in turn, activate Akt, a serine kinase with pleiotropic actions in several tissues (32). The activation of Akt-1/PKB is accompanied by an increase in its serine and threonine phosphorylation (22). Thus, the increase in the association between IRS-1 or IRS-2 and PI 3-kinase, and increased PI 3-kinase activity after leptin or insulin

infusion in the hypothalamus of exercised rats may play a role in the increased responsiveness to leptin and insulin in these animals.

Selective impairment of leptin and insulin signaling through the PI 3-kinase pathway in the hypothalamus could be pathophysiologically important in the development of obesity. Recent studies have shown that the activation of the PI 3-kinase pathway could be involved in the anorexigenic effect of insulin or leptin (14; 33; 34). Our findings, in a model of exercise, are relevant since insulin-induced tyrosine phosphorylation of IRSs and PI 3-kinase activity are reduced in the hypothalamus of different animal models of obesity (14; 35; 36). Thus, exercise training may be one therapeutic strategy to restore impaired leptin and insulin signal transduction in the hypothalamus of obese individuals.

In addition to the increased insulin and leptin sensitivity observed in the PI 3-kinase pathway, our data also provide evidence that there is an increase in leptin sensitivity in the JAK2/STAT3 pathway. Leptin activation of STAT3 requires the leptin receptor, which associates with and activates JAK2 in a ligand dependent manner (8; 9; 12; 37). One potential mediator of increased STAT3 activation in the hypothalamus of exercised rats is SOCS-3, a suppressor of cytokine signaling, expression. Forced expression of SOCS-3 in mammalian cells antagonizes leptin signaling, probably by binding and antagonizing JAK activity (38). Using Western blotting, we examined the expression of SOCS-3 in hypothalami of rats exercised. No significant differences were found between the two groups (data not shown). In addition, we found no significant difference in hypothalamic PTP1b expression and its association with JAK2 and OBR between the two groups (data not shown). Thus, a molecular basis for the observed increased in leptin's ability to activate STAT3 signaling after 1 day of exercise remains to be determined.

Perhaps the most striking finding was the reversal of exercise-induced increased hypothalamic insulin and leptin sensitivity by blocking the action of IL-6 action. These data are in accordance with earlier studies demonstrating that IL-6 treatment enhances energy expenditure in both rodents and humans (30; 39-41). It has been previously shown that IL-6 treatment stimulates energy expenditure at the level of the brain in rodents (30; 40; 42), and it might be assumed that endogenous IL-6 also acts on the brain

during exercise. The IL-6 exerting this effect during exercise could be produced by the brain itself, which has been shown to have increased IL-6 production during exercise (43). Alternatively, the large quantities of endocrine IL-6 produced from working skeletal muscle (44) might reach appropriate sites in the brain (23).

Numerous biologic responses of different cell types are induced by IL-6, which activates STAT3 and Ras-ERK1/2 via JAKS, and the balance of activation of both pathways is considered to direct the cell fate in response to IL-6 (45). The cross-talk of signals mediated by a cytokine and growth factor has been previously reported in the case of the phosphorylation of tyrosine kinase receptors by the growth hormone–activated JAK2 (46). This suggests that the IL-6–induced activation of JAK2 is involved in the activation of insulin and leptin receptor–mediated signals in rat hypothalamus. Conversely, it has been reported that the PI 3-kinase and Akt pathways may be activated via gp130 recruitment of adaptor molecules to create binding sites to the SH2 domain of the p85 subunit of PI 3-kinase (47).

In the present study, using an *in vivo* approach, we saw a synergistic effect of IL-6 on the insulin-stimulated tyrosine phosphorylation of the insulin receptor, IRS-1, and on the serine phosphorylation of PKB/Akt in rat hypothalamus. These results are clearly different from the findings of Senn *et al.* (48) in HepG2 cells and may indicate that the induction of SOCS-3 by IL-6 either follows a different time course in fat cells or that the major effect of IL-6 is exerted through other mechanisms such as the transcriptional regulation identified in the present work. However, the recent finding (49) that a high IL-6 infusion for 2 h in rats did not reduce the insulin effect during a euglycemic clamp, clearly supports the theory that any acute inhibitory effects of IL-6, mediated through a transient activation of SOCS-3, are of less importance for whole body insulin sensitivity. Similar results have recently been reported in man (50). Since IL-6 has been shown to interfere with insulin-signaling pathways in the liver and adipocytes in an inhibitory manner and to reduce insulin-stimulated glycogen synthesis in hepatocytes, the cross talk of IL-6 with insulin-signaling pathways appears to be tissue-specific.

The present study provides direct measurements of leptin and insulin signaling in the hypothalamus, and documents increased sensitivity to these hormones in the

hypothalamus of exercised rats in an IL-6 dependent manner. These findings provide support for the hypothesis that exercise could have appetite-suppressive actions mediated by the hypothalamus.

ACKNOWLEDGMENTS

These studies were supported by grants from Fundacão de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de desenvolvimento científico e tecnológico (CNPq). We are indebted to Dr. Nicola Conran for English language editing.

REFERENCES

1. Richard D, Lachance P, Deshaies Y: Effects of exercise-rest cycles on energy balance in rats. *Am J Physiol* 256:R886-891, 1989

2. Richard D, Rivest S: The role of exercise in thermogenesis and energy balance. *Can J Physiol Pharmacol* 67:402-409, 1989

3. Rivest S, Landry J, Richard D: Effect of exercise training on energy balance of orchidectomized rats. *Am J Physiol* 257:R550-555, 1989

4. Wing RR, Hill JO: Successful weight loss maintenance. *Annu Rev Nutr* 21:323-341, 2001

5. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM: Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269:543-546, 1995

6. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P: Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 269:546-549, 1995

7. Vaisse C, Halaas JL, Horvath CM, Darnell JE, Jr., Stoffel M, Friedman JM: Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice. *Nat Genet* 14:95-97, 1996

8. Ghilardi N, Ziegler S, Wiestner A, Stoffel R, Heim MH, Skoda RC: Defective STAT signaling by the leptin receptor in diabetic mice. *Proc Natl Acad Sci U S A* 93:6231-6235, 1996

9. Ghilardi N, Skoda RC: The leptin receptor activates janus kinase 2 and signals for proliferation in a factor-dependent cell line. *Mol Endocrinol* 11:393-399, 1997

10. Carvalheira JB, Siloto RM, Ignacchitti I, Brenelli SL, Carvalho CR, Leite A, Velloso LA, Gontijo JA, Saad MJ: Insulin modulates leptin-induced STAT3 activation in rat hypothalamus. *FEBS Lett* 500:119-124, 2001

11. Carvalheira JB, Ribeiro EB, Folli F, Velloso LA, Saad MJ: Interaction between leptin and insulin signaling pathways differentially affects JAK-STAT and PI 3-kinase-mediated signaling in rat liver. *Biol Chem* 384:151-159, 2003

12. Bjorbaek C, Uotani S, da Silva B, Flier JS: Divergent signaling capacities of the long and short isoforms of the leptin receptor. *J Biol Chem* 272:32686-32695, 1997

13. Kellerer M, Koch M, Metzinger E, Mushack J, Capp E, Haring HU: Leptin activates PI-3 kinase in C2C12 myotubes via janus kinase-2 (JAK-2) and insulin receptor substrate-2 (IRS-2) dependent pathways. *Diabetologia* 40:1358-1362, 1997

14. Carvalheira JB, Ribeiro EB, Araujo EP, Guimaraes RB, Telles MM, Torsoni M, Gontijo JA, Velloso LA, Saad MJ: Selective impairment of insulin signalling in the hypothalamus of obese Zucker rats. *Diabetologia* 46:1629-1640, 2003

15. Sun XJ, Rothenberg P, Kahn CR, Backer JM, Araki E, Wilden PA, Cahill DA, Goldstein BJ, White MF: Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. *Nature* 352:73-77, 1991

16. Lavan BE, Lane WS, Lienhard GE: The 60-kDa phosphotyrosine protein in insulin-treated adipocytes is a new member of the insulin receptor substrate family. *J Biol Chem* 272:11439-11443, 1997

17. Folli F, Saad MJ, Backer JM, Kahn CR: Insulin stimulation of phosphatidylinositol 3-kinase activity and association with insulin receptor substrate 1 in liver and muscle of the intact rat. *J Biol Chem* 267:22171-22177, 1992

18. Saad MJ, Folli F, Kahn JA, Kahn CR: Modulation of insulin receptor, insulin receptor substrate-1, and phosphatidylinositol 3-kinase in liver and muscle of dexamethasone-treated rats. *J Clin Invest* 92:2065-2072, 1993

19. Kuhne MR, Pawson T, Lienhard GE, Feng GS: The insulin receptor substrate 1 associates with the SH2-containing phosphotyrosine phosphatase Syp. *J Biol Chem* 268:11479-11481, 1993

20. Skolnik EY, Lee CH, Batzer A, Vicentini LM, Zhou M, Daly R, Myers MJ, Jr., Backer JM, Ullrich A, White MF, et al.: The SH2/SH3 domain-containing protein GRB2 interacts with tyrosine-phosphorylated IRS1 and Shc: implications for insulin control of ras signalling. *Embo J* 12:1929-1936, 1993

21. Yamauchi K, Milarski KL, Saltiel AR, Pessin JE: Protein-tyrosine-phosphatase SHPTP2 is a required positive effector for insulin downstream signaling. *Proc Natl Acad Sci U S A* 92:664-668, 1995

22. Kobayashi T, Cohen P: Activation of serum- and glucocorticoid-regulated protein kinase by agonists that activate phosphatidylinositide 3-kinase is mediated by 3-phosphoinositide-dependent protein kinase-1 (PDK1) and PDK2. *Biochem J* 339 (Pt 2):319-328, 1999

23. Pedersen BK, Hoffman-Goetz L: Exercise and the immune system: regulation, integration, and adaptation. *Physiol Rev* 80:1055-1081, 2000

24. Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B, Klarlund Pedersen B: Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J Physiol* 529 Pt 1:237-242, 2000

25. Penkowa M, Keller C, Keller P, Jauffred S, Pedersen BK: Immunohistochemical detection of interleukin-6 in human skeletal muscle fibers following exercise. *Faseb J* 17:2166-2168, 2003

26. Lyngso D, Simonsen L, Bulow J: Metabolic effects of interleukin-6 in human splanchnic and adipose tissue. *J Physiol* 543:379-386, 2002

27. Keller C, Keller P, Marshal S, Pedersen BK: IL-6 gene expression in human adipose tissue in response to exercise--effect of carbohydrate ingestion. *J Physiol* 550:927-931, 2003

28. Keller P, Keller C, Carey AL, Jauffred S, Fischer CP, Steensberg A, Pedersen BK: Interleukin-6 production by contracting human skeletal muscle: autocrine regulation by IL-6. *Biochem Biophys Res Commun* 310:550-554, 2003

29. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, et al.: Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1:1155-1161, 1995

30. Wallenius V, Wallenius K, Ahren B, Rudling M, Carlsten H, Dickson SL, Ohlsson C, Jansson JO: Interleukin-6-deficient mice develop mature-onset obesity. *Nat Med* 8:75-79, 2002

31. Chibalin AV, Yu M, Ryder JW, Song XM, Galuska D, Krook A, Wallberg-Henriksson H, Zierath JR: Exercise-induced changes in expression and activity of proteins involved in insulin signal transduction in skeletal muscle: differential effects on insulin-receptor substrates 1 and 2. *Proc Natl Acad Sci U S A* 97:38-43, 2000

32. Franke TF, Kaplan DR, Cantley LC, Toker A: Direct regulation of the Akt proto-oncogene product by phosphatidylinositol-3,4-bisphosphate. *Science* 275:665-668, 1997

33. Niswender KD, Morton GJ, Stearns WH, Rhodes CJ, Myers MG, Jr., Schwartz MW: Intracellular signalling. Key enzyme in leptin-induced anorexia. *Nature* 413:794-795, 2001

34. Niswender KD, Morrison CD, Clegg DJ, Olson R, Baskin DG, Myers MG, Jr., Seeley RJ, Schwartz MW: Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus: a key mediator of insulin-induced anorexia. *Diabetes* 52:227-231, 2003

35. De Souza CT, Araujo EP, Bordin S, Ashimine R, Zollner RL, Boschero AC, Saad MJ, Velloso LA: Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. *Endocrinology* 146:4192-4199, 2005

36. Prada PO, Zecchin HG, Gasparetti AL, Torsoni MA, Ueno M, Hirata AE, Corezola do Amaral ME, Hoer NF, Boschero AC, Saad MJ: Western diet modulates insulin signaling, c-Jun N-terminal kinase activity, and insulin receptor substrate-1ser307 phosphorylation in a tissue-specific fashion. *Endocrinology* 146:1576-1587, 2005

37. Baumann H, Morella KK, White DW, Dembski M, Bailon PS, Kim H, Lai CF, Tartaglia LA: The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. *Proc Natl Acad Sci U S A* 93:8374-8378, 1996

38. Bjorbaek C, El-Haschimi K, Frantz JD, Flier JS: The role of SOCS-3 in leptin signaling and leptin resistance. *J Biol Chem* 274:30059-30065, 1999

39. Stouthard JM, Romijn JA, Van der Poll T, Endert E, Klein S, Bakker PJ, Veenhof CH, Sauerwein HP: Endocrinologic and metabolic effects of interleukin-6 in humans. *Am J Physiol* 268:E813-819, 1995

40. Rothwell NJ, Busbridge NJ, Lefeuvre RA, Hardwick AJ, Gauldie J, Hopkins SJ: Interleukin-6 is a centrally acting endogenous pyrogen in the rat. *Can J Physiol Pharmacol* 69:1465-1469, 1991

41. Tsigos C, Papanicolaou DA, Defensor R, Mitsiadis CS, Kyrou I, Chrousos GP: Dose effects of recombinant human interleukin-6 on pituitary hormone secretion and energy expenditure. *Neuroendocrinology* 66:54-62, 1997

42. Li G, Klein RL, Matheny M, King MA, Meyer EM, Scarpace PJ: Induction of uncoupling protein 1 by central interleukin-6 gene delivery is dependent on sympathetic innervation of brown adipose tissue and underlies one mechanism of body weight reduction in rats. *Neuroscience* 115:879-889, 2002

43. Nybo L, Nielsen B, Pedersen BK, Moller K, Secher NH: Interleukin-6 release from the human brain during prolonged exercise. *J Physiol* 542:991-995, 2002

44. Ostrowski K, Rohde T, Zacho M, Asp S, Pedersen BK: Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *J Physiol* 508 (Pt 3):949-953, 1998

45. Ohtani T, Ishihara K, Atsumi T, Nishida K, Kaneko Y, Miyata T, Itoh S, Narimatsu M, Maeda H, Fukada T, Itoh M, Okano H, Hibi M, Hirano T: Dissection of signaling cascades through gp130 in vivo: reciprocal roles for STAT3- and SHP2-mediated signals in immune responses. *Immunity* 12:95-105, 2000

46. Yamauchi T, Ueki K, Tobe K, Tamemoto H, Sekine N, Wada M, Honjo M, Takahashi M, Takahashi T, Hirai H, Tushima T, Akanuma Y, Fujita T, Komuro I, Yazaki Y, Kadowaki T: Tyrosine phosphorylation of the EGF receptor by the kinase Jak2 is induced by growth hormone. *Nature* 390:91-96, 1997

47. Hideshima T, Nakamura N, Chauhan D, Anderson KC: Biologic sequelae of interleukin-6 induced PI3-K/Akt signaling in multiple myeloma. *Oncogene* 20:5991-6000, 2001

48. Senn JJ, Klover PJ, Nowak IA, Mooney RA: Interleukin-6 induces cellular insulin resistance in hepatocytes. *Diabetes* 51:3391-3399, 2002

49. Rotter Sopasakis V, Larsson BM, Johansson A, Holmang A, Smith U: Short-term infusion of interleukin-6 does not induce insulin resistance in vivo or impair insulin signalling in rats. *Diabetologia* 47:1879-1887, 2004

50. Steensberg A, Fischer CP, Sacchetti M, Keller C, Osada T, Schjerling P, van Hall G, Febbraio MA, Pedersen BK: Acute interleukin-6 administration does not impair muscle glucose uptake or whole-body glucose disposal in healthy humans. *J Physiol* 548:631-638, 2003

FIGURE LEGENDS

Figure 1. Physiological characteristics of control and exercised rats. Effects of exercise on plasma glucose concentration (ng/dl) (a), plasma insulin concentration (nmol/ml) (b) and plasma leptin concentration (ng/ml) (c). Data are expressed as means ± SEM, n=12. * p <0.01

Figure 2. Leptin inhibition of the 12-h cumulative food intake and leptin signaling in the hypothalamus of control and exercised rats. Vehicle (-) or leptin (+) was injected i.c.v. after a 6-h session of exercise and rats were immediately exposed to food for 12h. Data are expressed as means ± SEM of 8-14 animals per group (a). Tissue extracts were immunoprecipitated (IP, immunoprecipitation) with anti-OBR and anti JAK2 and immunoblotted (IB, immunoblotting) with antiphosphotyrosine antibody (pY) (b, c-*upper panel*). Whole-tissue extracts were immunoblotted with pSTAT3 antibody (d-*upper panel*). Stripped membranes were reblotted with anti-OBR, anti-JAK2 and anti-STAT3 antibodies (b, c, d-*lower panel*). The results of scanning densitometry (n=6) were expressed as arbitrary units. *Columns* and *bars* represent the mean ± SEM. *, p <0.05, leptin control *vs* leptin exercise. #, p <0.05, leptin control *vs* control.

Figure 3. Leptin signaling in the hypothalamus of control and exercised rats. Hypothalami extracts from rats injected with vehicle (-) or leptin (+) were prepared as decribed in *Materials and Methods*. Tissue extracts were immunoprecipitated (IP, immunoprecipitation) with anti-IRS-1 and anti-IRS-2 antibodies and immunoblotted (IB, immunoblotting) with antiphosphotyrosine (pY) (a, c-*upper panel*), anti-PI 3-kinase (a, c-*middle panel*), anti-IRS-1 and anti-IRS-2 antibodies (a, c-*lower panel*). PI 3-kinase assays were performed as described. Fluorographs show the silica TLC plates of IRS-1 or IRS-2 associated PI 3-kinase activity (b, d). PIP indicates the migration position of phosphatidylinositol 3–phosphate. The results of scanning densitometry (n=6) were expressed as arbitrary units. *Columns* and *bars* represent the mean ± SEM. *, p <0.05, leptin control *vs* leptin exercise.

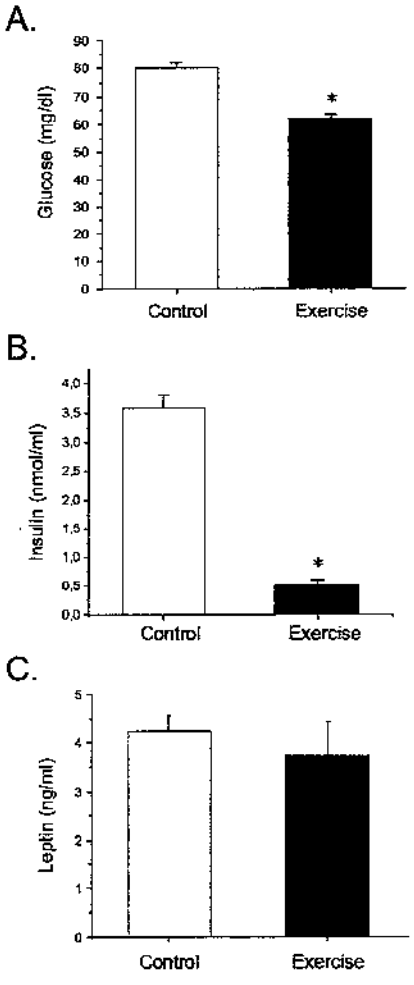
Figure 4. Insulin inhibition of the 12-h cumulative food intake and insulin signaling in the hypothalamus of control and exercised rats. Vehicle or insulin was injected i.c.v. after a session of 6-h exercise and rats were immediately exposed to food for 12h. Data are expressed as means ± SEM of 8-14 animals per group (a). Tissue extracts were immunoprecipitated (IP, immunoprecipitation) with anti-IR antibody and immunoblotted (IB, immunoblotting) with antiphosphotyrosine antibody (pY) (b-*upper panel*) and anti-IR antibody (b-*lower panel*). Tissue extracts were also immunoprecipitated with anti-IRS-1 and anti-IRS-2 antibodies and immunoblotted with antiphosphotyrosine (c, d-*upper panels*), anti-PI 3-kinase (c, d-*middle* panels), anti-IRS-1 or anti-IRS-2 antibodies (c, d-*lower panels*). The results of scanning densitometry (n=6) were expressed as arbitrary units. *Columns* and *bars* represent the mean ± SEM. *, $p < 0.05$, insulin control *vs* insulin exercise. #, $p < 0.05$, insulin control *vs* control.

Figure 5. Insulin signaling in the hypothalamus of control and exercised rats. Hypothalami from rats injected with vehicle (-) or insulin (+) were prepared as described in *Materials and Methods*. Fluorographs show the silica TLC plates of IRS-1 or IRS-2 associated PI 3-kinase activity. PIP indicates the migration position of phosphatidylinositol 3–phosphate (a, b).Whole tissue extracts immunoblotted (IB, immunoblotting) with anti-phospho Akt (c-*upper panel*) and with anti-Akt antibodies (c-lower panel). The results of scanning densitometry (n=6) were expressed as arbitrary units. *Columns* and *bars* represent the mean ± SEM. *, $p < 0.05$, insulin control *vs* insulin exercise.

Figure 6. Blockade of leptin and insulin-induced inhibition of food intake by anti-IL-6. Hypothalami from rats were prepared as described in *Materials and Methods*. Tissue extracts from control and exercised rats were immunoblotted with anti-IL-6 antibody (a). Leptin and insulin were injected i.c.v. in control rats, exercised rats and exercised rats pre-treated with anti-IL-6 and the animals were immediately exposed to food for 12h. Data are expressed as means ± SEM of 8-14 animals per group (b, c). Tissue extracts from control rats, exercised rats and exercised rats pretreated with anti-IL-6 were treated with leptin and immunoprecipitated (IP, immunoprecipitation) with anti-JAK2 antibody and immunoblotted (IB, immunoblotting) with antiphosphotyrosine antibody (d-*upper panel*). Whole tissue extracts were immunoblotted with anti-phospho STAT3

antibody (d-*lower panel*). Hypothalami tissues extracts from control, exercised and exercised plus anti-IL-6 rats were treated with insulin and immunoprecipitated with anti-IR antibody and immunoblotted with antiphosphotyrosine antibody (e-*upper panel*). Whole tissue extracts were immunoblotted with anti-phosphoserine Akt antibody (e-*lower panel*). The results of scanning densitometry (n=6) are expressed as arbitrary units. *Columns* and *bars* represent the mean ± SEM. *, $p < 0.05$, insulin control *vs* insulin exercise.

Fig. 1


A.
Glucose (mg/dl)
90
80
70
60
50
40
30
20
10
0
*
Control
Exercise
B.
Insulin (nmol/ml)
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
*
Control
Exercise
C.
Leptin (ng/ml)
5
4
3
2
1
0
Control
Exercise

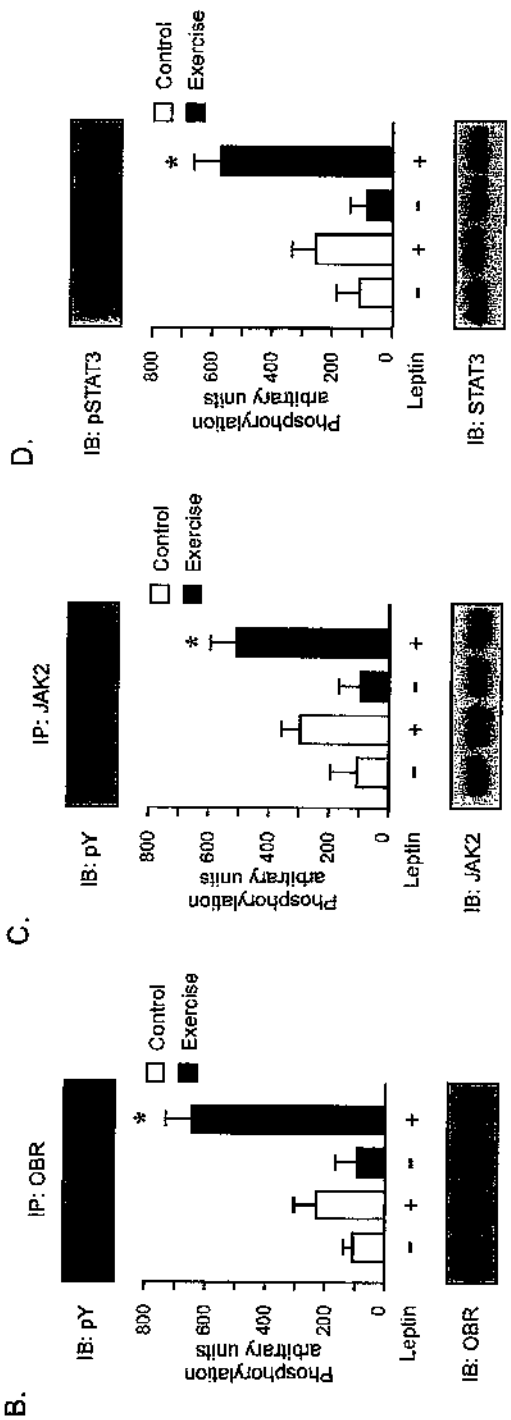

A.
Food intake (g)
16
12
8
4
0
Leptin
- + - +
#
*
Control
Exercise
B.
IP: OBR
IB: pY
IB: OBR
Phosphorylation arbitrary units
800
600
400
200
0
Leptin
- + - +
*
Control
Exercise
C.
IP: JAK2
IB: pY
IB: JAK2
Phosphorylation arbitrary units
800
600
400
200
0
Leptin
- + - +
*
Control
Exercise
D.
IB: pSTAT3
IB: STAT3
Phosphorylation arbitrary units
800
600
400
200
0
Leptin
- + - +
*
Control
Exercise

Fig. 2

Fig. 3

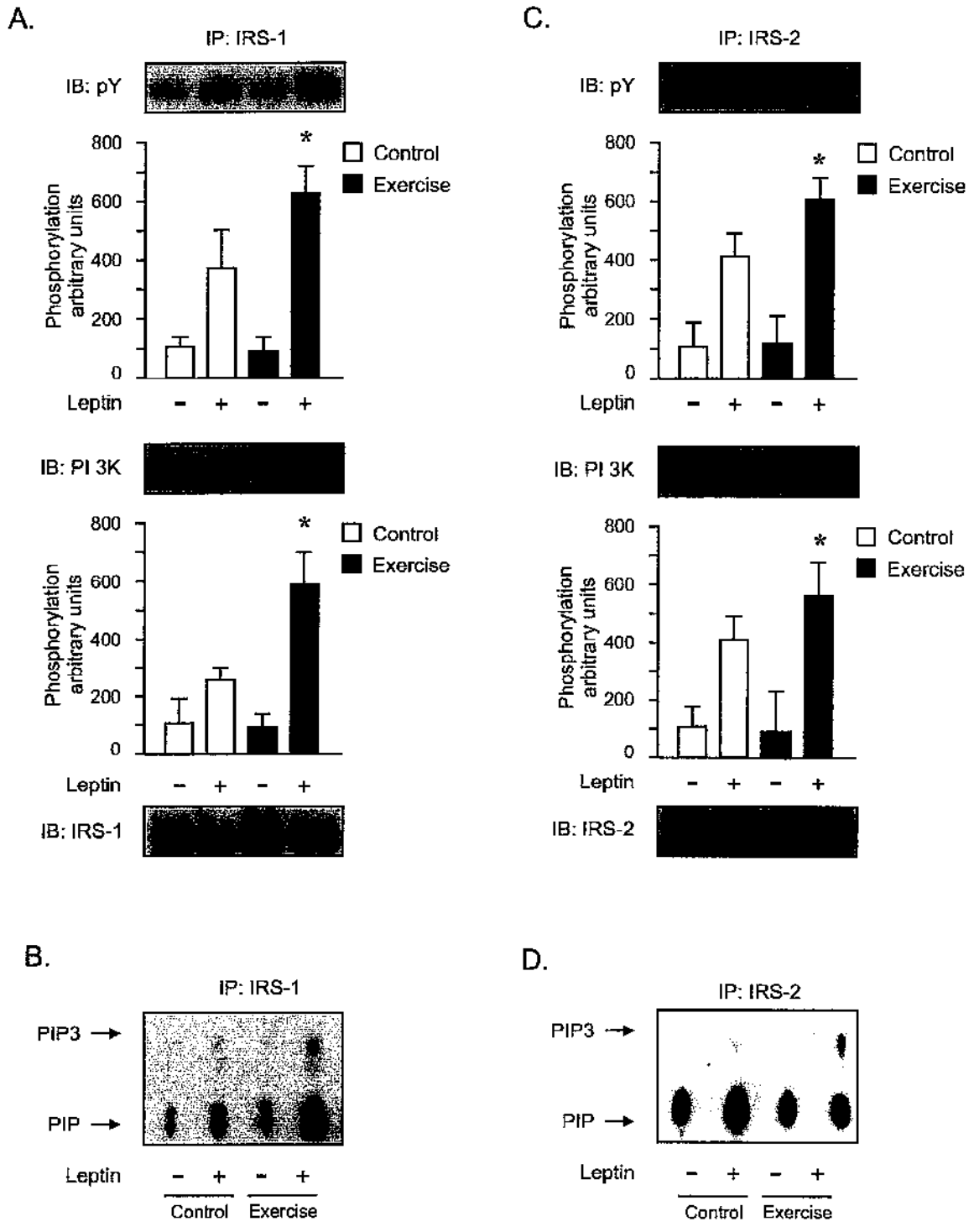

A.
IP: IRS-1
IB: pY
800
600
400
200
0
Phosphorylation arbitrary units
*
Control
Exercise
Leptin
- + - +
IB: PI 3K
IB: IRS-1
C.
IP: IRS-2
IB: IRS-2
B.
PIP3
PIP
Control
Exercise
D.

Fig. 4

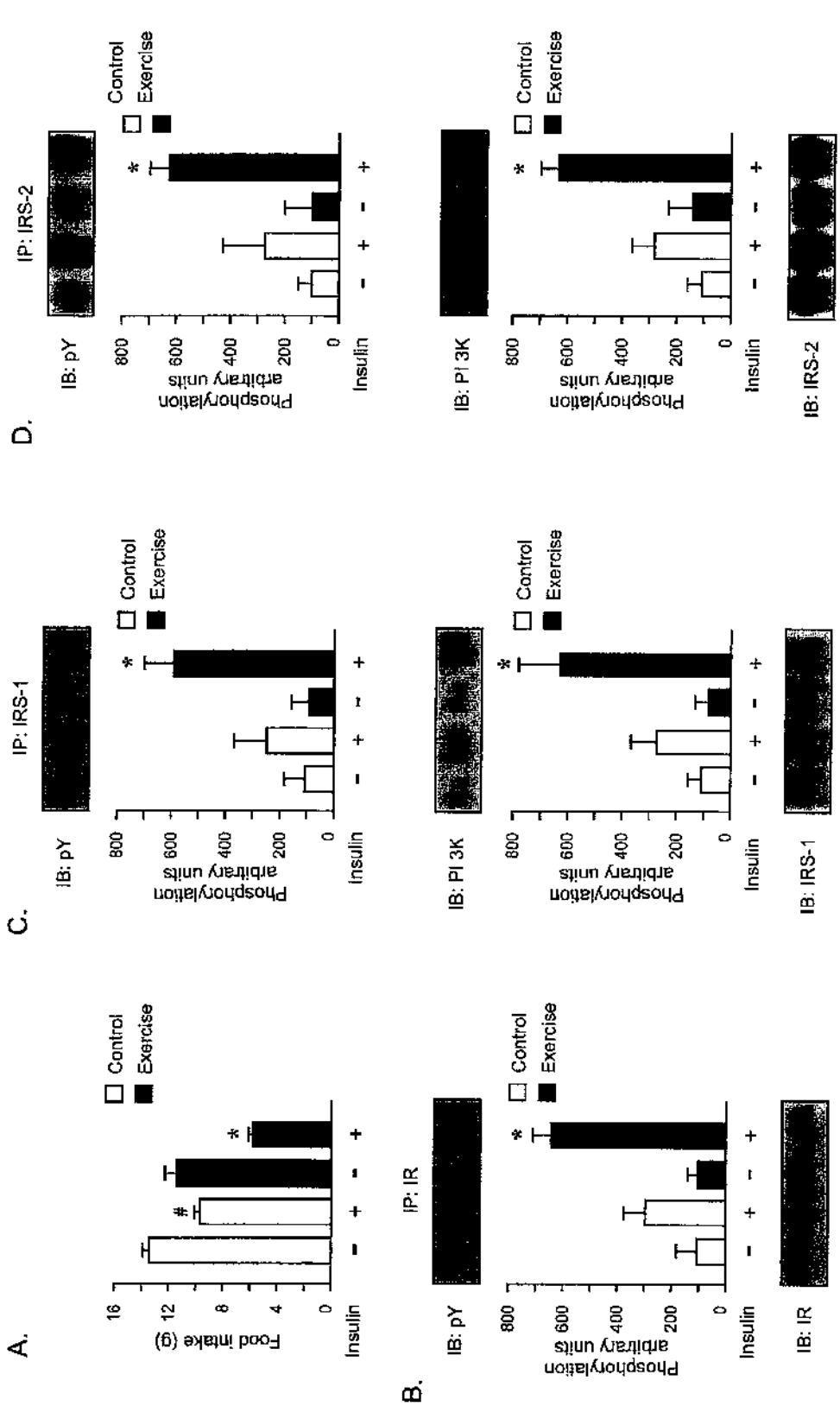

A.
Control
Exercise
Food intake (g)
16
12
8
4
0
Insulin
- + - +
#
*
B.
IP: IR
IB: pY
IB: IR
Phosphorylation arbitrary units
800
600
400
200
0
Insulin
- + - +
C.
IP: IRS-1
IB: pY
IB: PI 3K
Phosphorylation arbitrary units
800
600
400
200
0
Insulin
- + - +
D.
IP: IRS-2
IB: pY
IB: PI 3K
IB: IRS-2
Phosphorylation arbitrary units
800
600
400
200
0
Insulin
- + - +

Fig. 5

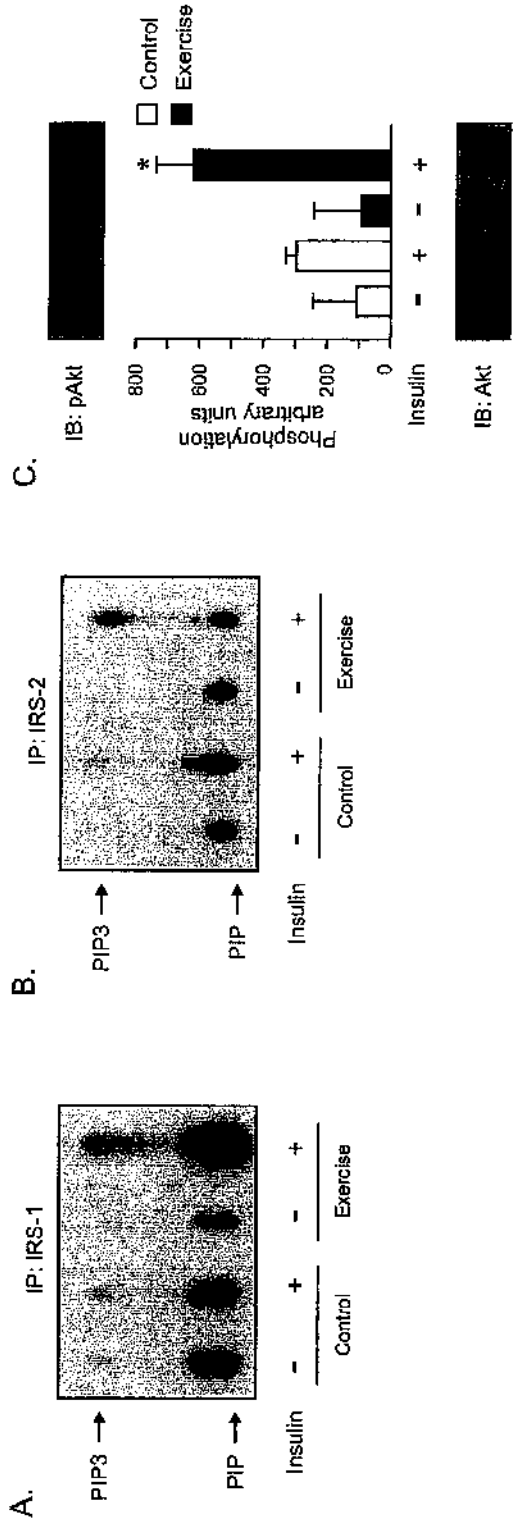
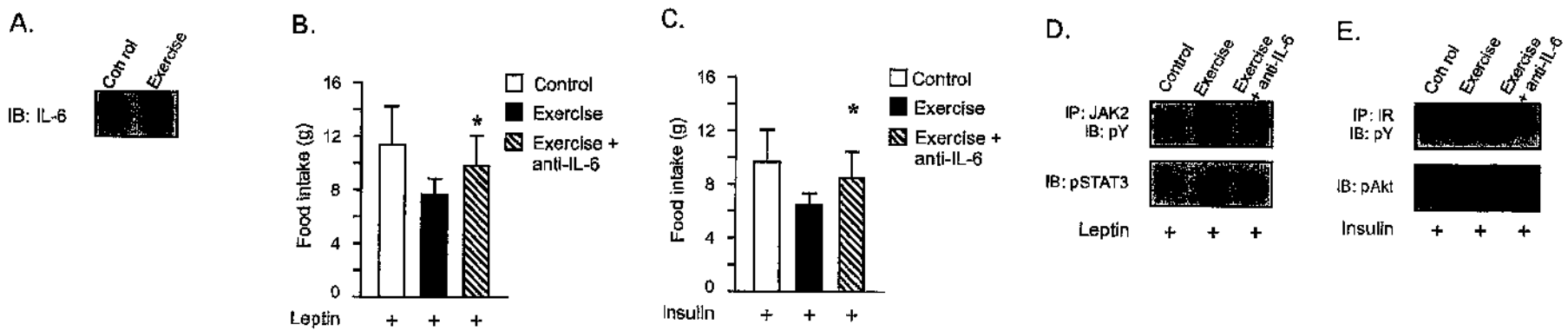

A.
IP: IRS-1
PIP3
PIP
Insulin
Control
Exercise
B.
IP: IRS-2
PIP3
PIP
Insulin
Control
Exercise
C.
IB: pAkt
Phosphorylation arbitrary units
800
600
400
200
0
Control
Exercise
Insulin
IB: Akt

Fig. 6


A.
Control
Exercise
IB: IL-6
B.
Food intake (g)
16
12
8
4
0
Leptin + + +
Control
Exercise
Exercise + anti-IL-6
*
C.
Food intake (g)
16
12
8
4
0
Insulin + + +
Control
Exercise
Exercise + anti-IL-6
*
D.
Control
Exercise
Exercise + anti-IL-6
IP: JAK2
IB: pY
IB: pSTAT3
Leptin + + +
E.
Control
Exercise
Exercise + anti-IL-6
IP: IR
IB: pY
IB: pAkt
Insulin + + +

4 - CONCLUSÃO

O presente estudo caracterizou a sinalização da leptina e insulina no hipotálamo, e documentou o aumento da sensibilidade do hipotalâmica para a ação destes hormônios, de forma dependente da interleucina-6 (IL-6) nos animais exercitados. Estes dados dão suporte à hipótese de que o exercício pode ter suas ações supressoras do apetite mediadas pelo hipotálamo.

5 - REFERÊNCIAS BIBLIOGRÁFICAS

Ahima, R. S.; Prabakaran, D.; Mantzoros, C.; Qu, D.; Lowell, B.; Maratos-Flier, E. et al. Role of leptin in the neuroendocrine response to fasting. **Nature,** 382: 250-2. 1996.

Araki, E.; Lipes, M. A.; Patti, M. E.; Bruning, J. C.; Haag, B., 3rd; Johnson, R. S. et al. Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. **Nature,** 372: 186-90. 1994.

Bado, A.; Levasseur, S.; Attoub, S.; Kermorgant, S.; Laigneau, J. P.; Bortoluzzi, M. N. et al. The stomach is a source of leptin. **Nature,** 394: 790-3. 1998.

Bandyopadhyay, G.; Standaert, M. L.; Zhao, L.; Yu, B.; Avignon, A.; Galloway, L. et al. Activation of protein kinase C (alpha, beta, and zeta) by insulin in 3T3/L1 cells. Transfection studies suggest a role for PKC-zeta in glucose transport. **J Biol Chem,** 272: 2551-8. 1997.

Baskin, D. G.; Breininger, J. F.Schwartz, M. W. Leptin receptor mRNA identifies a subpopulation of neuropeptide Y neurons activated by fasting in rat hypothalamus. **Diabetes,** 48: 828-33. 1999.

Baskin, D. G.; Wilcox, B. J.; Figlewicz, D. P.Dorsa, D. M. Insulin and insulin-like growth factors in the CNS. **Trends Neurosci,** 11: 107-11. 1988.

Bassuk, S. S. Manson, J. E. Epidemiological evidence for the role of physical activity in reducing risk of type 2 diabetes and cardiovascular disease. **Journal of Applied Physiology,** 99: 1193-204 2005.

Baumann, H.; Morella, K. K.; White, D. W.; Dembski, M.; Bailon, P. S.; Kim, H. et al. The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. **Proc Natl Acad Sci U S A,** 93: 8374-8. 1996.

Baura, G. D.; Foster, D. M.; Porte, D., Jr.; Kahn, S. E.; Bergman, R. N.; Cobelli, C. et al. Saturable transport of insulin from plasma into the central nervous system of dogs in vivo. A mechanism for regulated insulin delivery to the brain. **J Clin Invest,** 92: 1824-30. 1993.

Bi, S.; Scott, K. A.; Hyun, J.; Ladenheim, E. E.Moran, T. H. Running wheel activity prevents hyperphagia and obesity in Otsuka long-evans Tokushima Fatty rats: role of hypothalamic signaling. **Endocrinology,** 146: 1676-85 2005.

Bjorbaek, C.; Elmquist, J. K.; Frantz, J. D.; Shoelson, S. E.Flier, J. S. Identification of SOCS-3 as a potential mediator of central leptin resistance. **Mol Cell,** 1: 619-25. 1998.

Bovetto, S. Richard, D. Lesion of central nucleus of amygdala promotes fat gain without preventing effect of exercise on energy balance. **Am J Physiol,** 269: R781-6 1995.

Bruning, J. C.; Gautam, D.; Burks, D. J.; Gillette, J.; Schubert, M.; Orban, P. C. et al. Role of brain insulin receptor in control of body weight and reproduction. **Science,** 289: 2122-5. 2000.

Campfield, L. A.; Smith, F. J.; Guisez, Y.; Devos, R.Burn, P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. **Science,** 269: 546-9. 1995.

Caro, J. F.; Kolaczynski, J. W.; Nyce, M. R.; Ohannesian, J. P.; Opentanova, I.; Goldman, W. H. et al. Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. **Lancet,** 348: 159-61. 1996.

Carpenter, L. R.; Farruggella, T. J.; Symes, A.; Karow, M. L.; Yancopoulos, G. D.Stahl, N. Enhancing leptin response by preventing SH2-containing phosphatase 2 interaction with Ob receptor. **Proc Natl Acad Sci U S A,** 95: 6061-6. 1998.

Cheung, C. C.; Clifton, D. K.Steiner, R. A. Proopiomelanocortin neurons are direct targets for leptin in the hypothalamus. **Endocrinology,** 138: 4489-92. 1997.

Cline, G. W.; Petersen, K. F.; Krssak, M.; Shen, J.; Hundal, R. S.; Trajanoski, Z. et al. Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. **New England Journal of Medicine,** 341: 240-6 1999.

Coderre, L.; Kandror, K. V.; Vallega, G.Pilch, P. F. Identification and characterization of an exercise-sensitive pool of glucose transporters in skeletal muscle. **The Journal of Biological and Chemistry,** 270: 27584-8 1995.

Coleman, D. L. Effects of parabiosis of obese with diabetes and normal mice. **Diabetologia,** 9: 294-8. 1973.

Considine, R. V.; Sinha, M. K.; Heiman, M. L.; Kriauciunas, A.; Stephens, T. W.; Nyce, M. R. et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. **N Engl J Med,** 334: 292-5. 1996.

Czech, M. P. Corvera, S. Signaling mechanisms that regulate glucose transport. **J Biol Chem,** 274: 1865-8. 1999.

da Silva, B. A.; Bjorbaek, C.; Uotani, S.Flier, J. S. Functional properties of leptin receptor isoforms containing the gln-- >pro extracellular domain mutation of the fatty rat. **Endocrinology,** 139: 3681-90. 1998.

De Vos, P.; Saladin, R.; Auwerx, J.Staels, B. Induction of ob gene expression by corticosteroids is accompanied by body weight loss and reduced food intake. **J Biol Chem,** 270: 15958-61. 1995.

Dishman, R. K. Brain monoamines, exercise, and behavioral stress: animal models. **Med Sci Sports Exerc,** 29: 63-74 1997.

Douen, A. G.; Ramlal, T.; Rastogi, S.; Bilan, P. J.; Cartee, G. D.; Vranic, M. et al. Exercise induces recruitment of the "insulin-responsive glucose transporter". Evidence for distinct intracellular insulin- and exercise-recruitable transporter pools in skeletal muscle. **The Journal of Biological and Chemistry,** 265: 13427-30 1990.

Durbin, J. E.; Hackenmiller, R.; Simon, M. C.Levy, D. E. Targeted disruption of the mouse Stat1 gene results in compromised innate immunity to viral disease. **Cell,** 84: 443-50. 1996.

El-Haschimi, K.; Pierroz, D. D.; Hileman, S. M.; Bjorbaek, C.Flier, J. S. Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. **J Clin Invest,** 105: 1827-32. 2000.

Eriksson, J.; Tuominen, J.; Valle, T.; Sundberg, S.; Sovijarvi, A.; Lindholm, H. et al. Aerobic endurance exercise or circuit-type resistance training for individuals with impaired glucose tolerance? **Hormone and Metabolic Research,** 30: 37-41 1998.

Fan, W.; Boston, B. A.; Kesterson, R. A.; Hruby, V. J.Cone, R. D. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. **Nature,** 385: 165-8. 1997.

Farooqi, I. S.; Keogh, J. M.; Kamath, S.; Jones, S.; Gibson, W. T.; Trussell, R. et al. Partial leptin deficiency and human adiposity. **Nature,** 414: 34-5. 2001.

Frederich, R. C.; Hamann, A.; Anderson, S.; Lollmann, B.; Lowell, B. B.Flier, J. S. Leptin levels reflect body lipid content in mice: evidence for diet- induced resistance to leptin action. **Nat Med,** 1: 1311-4. 1995.

Friedman, J. M. Halaas, J. L. Leptin and the regulation of body weight in mammals. **Nature,** 395: 763-70. 1998.

Ghilardi, N.; Ziegler, S.; Wiestner, A.; Stoffel, R.; Heim, M. H.Skoda, R. C. Defective STAT signaling by the leptin receptor in diabetic mice. **Proc Natl Acad Sci U S A,** 93: 6231-5. 1996.

Goodyear, L. J. Kahn, B. B. Exercise, glucose transport, and insulin sensitivity. **Annual Review of Medicine,** 49: 235-61 1998.

Grundy, S. M. Barnett, J. P. Metabolic and health complications of obesity. **Dis Mon,** 36: 641-731. 1990.

Halaas, J. L.; Gajiwala, K. S.; Maffei, M.; Cohen, S. L.; Chait, B. T.; Rabinowitz, D. et al. Weight-reducing effects of the plasma protein encoded by the obese gene. **Science,** 269: 543-6. 1995.

Hathout, E. H.; Sharkey, J.; Racine, M.; Ahn, D.; Mace, J. W.Saad, M. F. Changes in plasma leptin during the treatment of diabetic ketoacidosis. **J Clin Endocrinol Metab,** 84: 4545-8. 1999.

Havel, P. J.; Uriu-Hare, J. Y.; Liu, T.; Stanhope, K. L.; Stern, J. S.; Keen, C. L. et al. Marked and rapid decreases of circulating leptin in streptozotocin diabetic rats: reversal by insulin. **Am J Physiol,** 274: R1482-91. 1998.

Hayashi, T.; Wojtaszewski, J. F.Goodyear, L. J. Exercise regulation of glucose transport in skeletal muscle. **The American Journal of Physiology,** 273: E1039-51 1997.

Heldin, C. H. Dimerization of cell surface receptors in signal transduction. **Cell,** 80: 213-23. 1995.

Henriksen, E. J. Invited review: Effects of acute exercise and exercise training on insulin resistance. **Journal Applied of Physiology,** 93: 788-96 2002.

Hevener, A. L.; Reichart, D.Olefsky, J. Exercise and thiazolidinedione therapy normalize insulin action in the obese Zucker fatty rat. **Diabetes,** 49: 2154-9 2000.

Howlett, K. F.; Sakamoto, K.; Hirshman, M. F.; Aschenbach, W. G.; Dow, M.; White, M. F. et al. Insulin signaling after exercise in insulin receptor substrate-2-deficient mice. **Diabetes,** 51: 479-83 2002.

James, D. E.; Burleigh, K. M.; Kraegen, E. W.Chisholm, D. J. Effect of acute exercise and prolonged training on insulin response to intravenous glucose in vivo in rat. **Journal of Applied Physiology,** 55: 1660-4 1983.

Kahn, S. E.; Prigeon, R. L.; McCulloch, D. K.; Boyko, E. J.; Bergman, R. N.; Schwartz, M. W. et al. Quantification of the relationship between insulin sensitivity and beta- cell function in human subjects. Evidence for a hyperbolic function. **Diabetes,** 42: 1663-72. 1993.

Kelly, J.; Alheid, G. F.; Newberg, A.Grossman, S. P. GABA stimulation and blockade in the hypothalamus and midbrain: effects on feeding and locomotor activity. **Pharmacol Biochem Behav,** 7: 537-41 1977.

Kennedy, G. C. Role of depot fat in hypothalamic control of food intake in rat. **Proc. R. Soc. Lond.,** 578- 592 1953.

Kennedy, J. W.; Hirshman, M. F.; Gervino, E. V.; Ocel, J. V.; Forse, R. A.; Hoenig, S. J. et al. Acute exercise induces GLUT4 translocation in skeletal muscle of normal human subjects and subjects with type 2 diabetes. **Diabetes,** 48: 1192-7 1999.

Kim, Y. B.; Nikoulina, S. E.; Ciaraldi, T. P.; Henry, R. R.Kahn, B. B. Normal insulin-dependent activation of Akt/protein kinase B, with diminished activation of phosphoinositide 3-kinase, in muscle in type 2 diabetes. **J Clin Invest,** 104: 733-41. 1999.

Kitamura, T.; Ogawa, W.; Sakaue, H.; Hino, Y.; Kuroda, S.; Takata, M. et al. Requirement for activation of the serine-threonine kinase Akt (protein kinase B) in insulin stimulation of protein synthesis but not of glucose transport. **Mol Cell Biol,** 18: 3708-17. 1998.

Knowler, W. C.; Barrett-Connor, E.; Fowler, S. E.; Hamman, R. F.; Lachin, J. M.; Walker, E. A. et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. **New England Journal of Medicine,** 346: 393-403 2002.

Kohn, A. D.; Summers, S. A.; Birnbaum, M. J.Roth, R. A. Expression of a constitutively active Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. **J Biol Chem,** 271: 31372-8. 1996.

Kopelman, P. G. Obesity as a medical problem. **Nature,** 404: 635-43. 2000.

Kotani, K.; Ogawa, W.; Matsumoto, M.; Kitamura, T.; Sakaue, H.; Hino, Y. et al. Requirement of atypical protein kinase clambda for insulin stimulation of glucose uptake but not for Akt activation in 3T3-L1 adipocytes. **Mol Cell Biol,** 18: 6971-82. 1998.

Kristensen, P.; Judge, M. E.; Thim, L.; Ribel, U.; Christjansen, K. N.; Wulff, B. S. et al. Hypothalamic CART is a new anorectic peptide regulated by leptin. **Nature,** 393: 72-6. 1998.

Kubo, K. Foley, J. E. Rate-limiting steps for insulin-mediated glucose uptake into perfused rat hindlimb. **The American Journal of Physiology,** 250: E100-2 1986.

Lee, G. H.; Proenca, R.; Montez, J. M.; Carroll, K. M.; Darvishzadeh, J. G.; Lee, J. I. et al. Abnormal splicing of the leptin receptor in diabetic mice. **Nature,** 379: 632-5. 1996.

Leedom, L. J. Meehan, W. P. The psychoneuroendocrinology of diabetes mellitus in rodents. **Psychoneuroendocrinology,** 14: 275-94 1989.

Leibowitz, S. F.; Roossin, P.Rosenn, M. Chronic norepinephrine injection into the hypothalamic paraventricular nucleus produces hyperphagia and increased body weight in the rat. **Pharmacol Biochem Behav,** 21: 801-8 1984.

Levin, B. E. Dunn-Meynell, A. A. Chronic exercise lowers the defended body weight gain and adiposity in diet-induced obese rats. **Am J Physiol Regul Integr Comp Physiol,** 286: R771-8 2004.

Levin, B. E.; Dunn-Meynell, A. A.; McMinn, J. E.; Alperovich, M.; Cunningham-Bussel, A.Chua, S. C., Jr. A new obesity-prone, glucose-intolerant rat strain (F.DIO). **Am J Physiol Regul Integr Comp Physiol,** 285: R1184-91 2003.

Licinio, J.; Mantzoros, C.; Negrao, A. B.; Cizza, G.; Wong, M. L.; Bongiorno, P. B. et al. Human leptin levels are pulsatile and inversely related to pituitary- adrenal function. **Nat Med,** 3: 575-9. 1997.

Luciano, E.; Carneiro, E. M.; Carvalho, C. R.; Carvalheira, J. B.; Peres, S. B.; Reis, M. A. et al. Endurance training improves responsiveness to insulin and modulates insulin signal transduction through the phosphatidylinositol 3-kinase/Akt-1 pathway. **European Journal of Endocrinology,** 147: 149-57 2002.

Lyons, W. E.; Mamounas, L. A.; Ricaurte, G. A.; Coppola, V.; Reid, S. W.; Bora, S. H. et al. Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. **Proc Natl Acad Sci U S A,** 96: 15239-44 1999.

Maffei, M.; Halaas, J.; Ravussin, E.; Pratley, R. E.; Lee, G. H.; Zhang, Y. et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. **Nat Med,** 1: 1155-61. 1995.

Masuzaki, H.; Ogawa, Y.; Sagawa, N.; Hosoda, K.; Matsumoto, T.; Mise, H. et al. Nonadipose tissue production of leptin: leptin as a novel placenta- derived hormone in humans. **Nat Med,** 3: 1029-33. 1997.

Meraz, M. A.; White, J. M.; Sheehan, K. C.; Bach, E. A.; Rodig, S. J.; Dighe, A. S. et al. Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway. **Cell,** 84: 431-42. 1996.

Mercer, J. G.; Hoggard, N.; Williams, L. M.; Lawrence, C. B.; Hannah, L. T.Trayhurn, P. Localization of leptin receptor mRNA and the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization. **FEBS Lett,** 387: 113-6. 1996.

Miller, W. C.; Koceja, D. M.Hamilton, E. J. A meta-analysis of the past 25 years of weight loss research using diet, exercise or diet plus exercise intervention. **Int J Obes Relat Metab Disord,** 21: 941-7 1997.

Montague, C. T.; Farooqi, I. S.; Whitehead, J. P.; Soos, M. A.; Rau, H.; Wareham, N. J. et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans. **Nature,** 387: 903-8. 1997.

Neeper, S. A.; Gomez-Pinilla, F.; Choi, J.Cotman, C. W. Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. **Brain Res,** 726: 49-56 1996.

Niswender, K. D.; Morton, G. J.; Stearns, W. H.; Rhodes, C. J.; Myers, M. G., Jr.Schwartz, M. W. Intracellular signalling. Key enzyme in leptin-induced anorexia. **Nature,** 413: 794-5. 2001.

O'Donovan, G.; Kearney, E. M.; Nevill, A. M.; Woolf-May, K.Bird, S. R. The effects of 24 weeks of moderate- or high-intensity exercise on insulin resistance. **European Journal of Applied Physiology,** 1-7 2005.

Pelleymounter, M. A.; Cullen, M. J.; Baker, M. B.; Hecht, R.; Winters, D.; Boone, T. et al. Effects of the obese gene product on body weight regulation in ob/ob mice. **Science,** 269: 540-3. 1995.

Polonsky, K. S.; Given, B. D.Van Cauter, E. Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. **J Clin Invest,** 81: 442-8. 1988.

Pronk, N. P.; Wing, R. R.Hamilton, E. J. Physical activity and long-term maintenance of weight loss. **Obes Res,** 2: 587-91 1994.

Rivest, S. Richard, D. Involvement of corticotropin-releasing factor in the anorexia induced by exercise. **Brain Res Bull,** 25: 169-72 1990.

Saladin, R.; De Vos, P.; Guerre-Millo, M.; Leturque, A.; Girard, J.; Staels, B. et al. Transient increase in obese gene expression after food intake or insulin administration. **Nature,** 377: 527-9. 1995.

Schwartz, M. W.; Prigeon, R. L.; Kahn, S. E.; Nicolson, M.; Moore, J.; Morawiecki, A. et al. Evidence that plasma leptin and insulin levels are associated with body adiposity via different mechanisms. **Diabetes Care,** 20: 1476-81. 1997.

Shimoda, K.; van Deursen, J.; Sangster, M. Y.; Sarawar, S. R.; Carson, R. T.; Tripp, R. A. et al. Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. **Nature,** 380: 630-3. 1996.

Shin, M. S.; Kim, H.; Chang, H. K.; Lee, T. H.; Jang, M. H.; Shin, M. C. et al. Treadmill exercise suppresses diabetes-induced increment of neuropeptide Y expression in the hypothalamus of rats. **Neurosci Lett,** 346: 157-60 2003.

Shutter, J. R.; Graham, M.; Kinsey, A. C.; Scully, S.; Luthy, R.Stark, K. L. Hypothalamic expression of ART, a novel gene related to agouti, is up- regulated in obese and diabetic mutant mice. **Genes Dev,** 11: 593-602. 1997.

Sipols, A. J.; Baskin, D. G.Schwartz, M. W. Effect of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. **Diabetes,** 44: 147-51. 1995.

Stephens, T. W.; Basinski, M.; Bristow, P. K.; Bue-Valleskey, J. M.; Burgett, S. G.; Craft, L. et al. The role of neuropeptide Y in the antiobesity action of the obese gene product. **Nature,** 377: 530-2. 1995.

Takeda, K.; Tanaka, T.; Shi, W.; Matsumoto, M.; Minami, M.; Kashiwamura, S. et al. Essential role of Stat6 in IL-4 signalling. **Nature,** 380: 627-30. 1996.

Tartaglia, L. A. The leptin receptor. **J Biol Chem,** 272: 6093-6. 1997.

Tartaglia, L. A.; Dembski, M.; Weng, X.; Deng, N.; Culpepper, J.; Devos, R. et al. Identification and expression cloning of a leptin receptor, OB-R. **Cell,** 83: 1263-71. 1995.

Teran-Garcia, M.; Rankinen, T.; Koza, R. A.; Rao, D. C.Bouchard, C. Endurance training-induced changes in insulin sensitivity and gene expression. **American Journal of Physiology. Endocrinology and Metabolism,** 288: E1168-78 2005.

Timofeeva, E.; Huang, Q.Richard, D. Effects of treadmill running on brain activation and the corticotropin-releasing hormone system. **Neuroendocrinology,** 77: 388-405 2003.

Vaisse, C.; Halaas, J. L.; Horvath, C. M.; Darnell, J. E., Jr.; Stoffel, M.Friedman, J. M. Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice. **Nat Genet,** 14: 95-7. 1996.

Waldbillig, R. J.; Bartness, T. J.Stanley, B. G. Increased food intake, body weight, and adiposity in rats after regional neurochemical depletion of serotonin. **J Comp Physiol Psychol,** 95: 391-405 1981.

Withers, D. J.; Gutierrez, J. S.; Towery, H.; Burks, D. J.; Ren, J. M.; Previs, S. et al. Disruption of IRS-2 causes type 2 diabetes in mice. **Nature,** 391: 900-4. 1998.

Wojtaszewski, J. F.; Higaki, Y.; Hirshman, M. F.; Michael, M. D.; Dufresne, S. D.; Kahn, C. R. et al. Exercise modulates postreceptor insulin signaling and glucose transport in muscle-specific insulin receptor knockout mice. **The Journal of Clinical Investigation,** 104: 1257-64 1999.

Woods, S. C.; Chavez, M.; Park, C. R.; Riedy, C.; Kaiyala, K.; Richardson, R. D. et al. The evaluation of insulin as a metabolic signal influencing behavior via the brain. **Neurosci Biobehav Rev,** 20: 139-44 1996.

Woods, S. C.; Lotter, E. C.; McKay, L. D.Porte, D., Jr. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. **Nature,** 282: 503-5. 1979.

Woods, S. C.; Porte, D., Jr.; Bobbioni, E.; Ionescu, E.; Sauter, J. F.; Rohner-Jeanrenaud, F. et al. Insulin: its relationship to the central nervous system and to the control of food intake and body weight. **Am J Clin Nutr,** 42: 1063-71. 1985.

Zhang, Y.; Proenca, R.; Maffei, M.; Barone, M.; Leopold, L.Friedman, J. M. Positional cloning of the mouse obese gene and its human homologue. **Nature,** 372: 425-32. 1994.