UNIVERSIDADE FEDERAL DE MATO GROSSO

FACULDADE DE NUTRIÇÃO

PROGRAMA DE PÓS GRADUAÇÃO EM BIOCIÊNCIAS

AVALIAÇÃO DO PAPEL DO GLUCAGON E DO AMPc NA PRODUÇÃO DE GLICOSE, GLICÓLISE E GLICOGENÓLISE NO FÍGADO DE RATOS ALIMENTADOS COM FARINHA DE SOJA

Jhulie Kalina Barrankievicz

Cuiabá-MT

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ALT – Alanina Aminotransferase

AMPc/cAMP – Adenosina Monofosfato Cíclico / Adenosina 3’5’-Monofosfato Cíclico

AMP – Adenosina Monofosfato

ANOVA – Análise de Variância

AST – Aspartato Aminotransferase

ATP – Adenosina Trifosfato

PFK - Fosfofrutoquinase

F-1,6-P_2 – Frutose-1,6-bifosfato

F-1,6-P_2ase – Frutose-1,6-bifosfatase

F-2,6-P_2 – Frutose-2,6-bifosfato

F-6-P – Frutose-6-fosfato

GF – Glicogênio Fosforilase

GLUT – Transportador de Glicose

G-6-P – Glicose-6-fosfato

G-6-Pase – Glicose-6-fosfatase

IR – Receptor de Insulina

IRS1 – Substrato de Receptor de Insulina 1

KHB – Tampão Krebs-Henseleit Bicarbonato

LSD – Diferença Mínima Significativa

mmol – Milomolar

PEPCK - Fosfoenolpiruvato Carboxiquinase

PKA – Proteína quinase A

T2DM – Diabetes Mellitus tipo 2

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Figure 2 – Glucose production (A), glycolysis (C) and glycogenolysis (E) stimulated by cAMP 5µM and the corresponding areas under curves (AUCs) (B, D, E) in livers of adult rats maintained on casein control (CC and LC), soybean flour (CS, LS) or low-protein (LL) diets after weaning. The livers were perfused as described in materials and methods. The values are expressed as the mean ± SD (n=8-10 rats). *Mean values were significantly different from the control rats (p<0.05; two way ANOVA). #Mean values were significantly different from the rats fed a casein diet (p<0.05, two-way ANOVA). Means with superscript minuscule letters are significantly different by one-way ANOVA followed by LSD test (p< 0.05).

RESUMO

A desnutrição nas fases iniciais do crescimento e desenvolvimento aumenta a susceptibilidade à intolerância à glicose ou diabetes mellitus tipo 2 durante a vida adulta e o consumo de alimentos à base de soja parece ser benéfico na prevenção e tratamento de doenças.a utilização da soja é indicada como sendo útil na prevenção dessas doenças. Devido à sua recomendação, nossos estudos são focados na ação da soja como método para recuperação de modelo animal de restrição protéica durante o início da vida. Entretanto, nossos resultados estão em conflito com a visão do potencial efeito positivo da dieta à base de soja, pois resultou em um nível reduzido de glicogênio hepático e aumento sérico de ALT. Assim, considerando estes resultados, investigamos se a dieta e/ou o estado nutricional poderia afetar a ação do glucagon e AMPc na produção de glicose, glicólise e glicogenólise pela técnica de perfusão hepática. Ratos macho, crias de mães alimentadas com 17% ou 6% de proteína (caseína) durante a gestação e a lactação foram mantidos com dieta a 17% de caseína (CC e LC) ou à base de farinha soja (CS e LS) e com 6% de caseína (grupo LL) até 90 dias de vida. Os ratos mantidos com dieta à base de farinha de soja tiveram peso corporal e consumo alimentar absoluto menores em relação aos animais mantidos com dieta à base de caseína. O fígado dos grupos recuperados (LC e LS) respondeu de forma mais eficaz à ação do AMPc em comparação ao grupo LL, sugerindo maior sensibilidade ao agente glicolítico. Através da perfusão com AMPc, os grupos alimentados com dieta à base de soja apresentaram aumento da produção de glicose, maior inibição da glicólise sem aumento significativo na glicogenólise. Assim, sugerimos que a produção de glicose pelo fígado dos grupos alimentados com soja seja, pelo menos em parte, reciclada para ser utilizada na síntese de glicogênio como forma de garantir algum teor de glicogênio em relação aos grupos alimentados com dieta de controle.

Palavras chaves: Produção de glicose; Glicólise; Glicogenólise; Soja; Restrição proteica; Recuperação; Ratos.

1. INTRODUÇÃO

A manutenção da glicose plasmática dentro de uma estreita faixa de variação, a despeito das grandes flutuações no seu fornecimento e na sua demanda, é imprescindível devido aos efeitos decorrentes da hipo e hiperglicemias. Em condições normais, os níveis glicêmicos de um indivíduo em jejum, pela manhã, é de aproximadamente 90 mg/dl, atingindo concentração máxima de 165 mg/dl após as refeições e concentração mínima de 55 mg/dl durante o exercício ou jejum moderado (60 horas) (Rizza *et al*. 1980, Wahren *et al*. 1978, Consoli *et al*. 1987). Concentrações anormais de glicose plasmática podem causar danos ao organismo, por exemplo no cérebro, onde a glicose é a principal fonte de energia. Assim sua diminuição (hipoglicemia) pode levar a deficiência da função cerebral e morte, já seu aumento (hiperglicemia), um sintoma clínico de diabetes, aumenta o risco de várias complicações macro e microvasculares (Sharabi *et al*. 2015).

Manter a homeostase glicêmica requer um equilíbrio entre a entrada e a remoção da glicose da circulação (Gerich, 1993). Entre os tecidos que contribuem para a manutenção dos níveis normais de glicose no sangue estão o fígado (remove 34% de glicose), músculo e tecido adiposo (removem juntos 33% de glicose), cérebro, rim e células vermelhas do sangue (removem juntos 33% de glicose) (Moore *et al*. 2012). A captação de glicose pelo músculo, fígado e tecido adiposo é influenciada pelas concentrações de insulina e glicose plasmáticas, enquanto a captação de glicose pelo cérebro é independente de insulina (Gerich, 1993). A insulina é responsável por regular as concentrações dos transportadores de glicose (GLUT), pois promove sua síntese e os mobiliza do citoplasma para a membrana celular (Cushman & Wardzala, 1980).

A glicose captada pelas células segue basicamente três destinos: 1- estocada como glicogênio ou triglicerídeos; 2- oxidada a CO_2 e 3- convertida a lactato (ou alanina) que é subsequentemente liberado na circulação. A proporção de glicose que é direcionada para cada uma dessas vias, varia entre os tecidos e depende do estado metabólico, do meio hormonal e da presença de substratos alternativos, à exemplo dos ácidos graxos livres (Felig 1973; Randle *et al*. 1988). No cérebro, quase toda glicose captada é completamente

oxidada, enquanto que no músculo, células sanguíneas, pele, rim e trato gastrointestinal a maior parte da glicose captada sofre glicólise e é reciclada de volta para o fígado como lactato ou alanina (Gerich, 1993).

O fígado desempenha um importante papel na homeostase da glicose devido a sua habilidade em estocar glicose na forma de glicogênio (glicogênese), produzir glicose a partir da quebra do glicogênio (glicogenólise) ou sintetizar glicose a partir de outros substratos disponíveis (gliconeogênese) (Lin & Accili 2011, Roden & Bernroider 2003).

Insulina e glucagon são os hormônios que se contraregulam e coordenam as respostas dos tecidos periféricos para controlar as taxas de utilização e produção da glicose, mantendo a glicemia dentro da faixa de normalidade. A resistência desses tecidos à insulina é o principal fator que prejudica a homeostase glicêmica levando a uma hiperglicemia e consequentemete ao desenvolvimento de diabetes mellitus tipo 2 (T2DM) (Samuel & Shulman 2012). O glucagon desempenha funções importantes na homeostase glicêmica normal e em anormalidades metabólicas, particularmente o diabetes (Miller & Birnbaum 2016).

A insulina é secretada pelas células β pancreáticas, em resposta ao aumento dos níveis de glicose circulante após as refeições, e exerce papel fundamental no metabolismo da glicose, através da captação de glicose e seu armazenamento na forma de glicogênio hepático (glicogênese), lipogênese (Samuel & Shulman 2012) e reduzindo a liberação de glicose hepática (glicogenólise) (Carvalheira *et al*. 2002). Esses processos são desencadeados pela ligação da insulina ao seu receptor de membrana, que é altamente regulado e específico (Pessin & Saltiel 2000).

O hormônio glucagon é conhecido como regulador positivo do sistema de produção de glicose, pois sua principal função consiste em aumentar a concentração de glicose no sangue, através da glicogenólise e gliconeogênese hepáticas, por este motivo é denominado antagonista da insulina (McArdle, Katch & Katch, 1988). O glucagon é secretado pelas células α pancreáticas em resposta a mudança na concentração sanguínea de glicose, aminoácidos ou insulina (Miller & Birnbaum 2016). O principal papel fisiológico do glucagon é estimular a produção hepática de glicose reestabelecendo a glicemia. Para isso, o glucagon se liga a subunidades extracelulares do receptor de glucagon,

o que resulta em mudanças conformacionais ativando as proteínas G acopladas. Duas classes de proteínas G estão envolvidas na transdução de sinal do receptor de glucagon, $G_s\alpha$ e G_q (Jiang & Zhang 2003).

A ativação de $G_s\alpha$ por meio da ligação do glucagon ao seu receptor na superfície da membrana, aumenta os níveis intracelulares de adenosina 3'5'-monofosfato cíclico (AMPc) via adenilato ciclase leva a ativação de adenilato ciclase, com subsequente ativação da proteína quinase A (PKA). PKA fosforila e ativa glicogênio fosforilase (GF) que, por sua vez, fosforila o glicogênio resultando na produção de glicose-6-fosfato (G-6-P). A G-6-P é convertida em glicose pela ação da enzima glicose-6-fosfatase (G-6-Pase), aumentando a liberação de glicose a partir da quebra do glicogênio (glicogenólise) (Johnson *et al*. 1997; Krebs, 1981). O glucagon também inibe a atividade da glicogênio sintase no fígado, assim, a glicogênio sintase inativada reduz a síntese de glicogênio e consequentemente aumenta a disponibilidade de glicose no sangue (Roach,1990). Desta forma, a sinalização do glucagon promove glicogenólise e ao mesmo tempo inibe a síntese de glicogênio (glicogênese) no fígado.

Além de afetar o metabolismo do glicogênio, o glucagon regula a glicemia por afetar o metabolismo da glicose, diminuindo a glicólise. A glicólise consiste na quebra da glicose formando piruvato (que pode ser convertido em lactato). No primeiro passo da via glicolítica, a glicose captada pela célula é fosforilada a glicose-6-fosfato (G-6-P) (reação catalisada pela enzima glicoquinase no fígado), que em seguida pode ser convertida a glicogênio ou a frutose-6-fosfato (F-6-P). A F-6-P é convertida a frutose-1,6-bifosfato (F-1,6-P_2), reação catalisada pela fosfofrutoquinase (PFK). O próximo passo envolve a conversão de F-1,6-P_2 a gliceraldeído-3-fosfato via aldolase e conversão desses substratos para 1,3-difosfoglicerato via gliceraldeído-3-fosfato desidrogenase com consequente formação de fosfoenolpiruvato. O último passo da glicólise é a conversão de fosfoenolpiruvato a piruvato, reação catalisada pela enzima piruvato quinase. O piruvato pode ser reduzido a lactato pela lactato desidrogenase ou seguir para a mitocôndria onde entrará no ciclo de Krebs (Gerich, 1993).

A glicólise e a gliconeogênese são reguladas de maneira separada e recíproca, quando uma delas está ativa, a outra está desacelerada, devido

ação da frutose-2,6-bifosfato (F-2,6-P_2), que ativa a PFK, enzima que cataliza a fosforilação da F-6-P a F-1,6-P_2 e inibe a frutose-1,6-bifosfatase (F-1,6-P_2ase), que faz a reação inversa, desfosforilação da F-1,6-P_2 a F-6-P, assim a F-2,6-P_2 controla a glicólise e gliconeogênese, respectivamete. A formação da F-2,6-P_2 é inibida por AMPc que é estimulada pelo glucagon. O resultado da redução intrahepática de F-2,6-P_2 levaria a inibição da glicólise e promoveria a gliconeogênese (Pilkis, El-Maghrabi & Claus 1990).

Numerosos estudos *in vivo* ou *in vitro* de perfusão em fígado de ratos, demonstraram que o glucagon estimula a produção de glicose, resultando em aumentos da glicogenólise e gliconeogênese e diminuição da glicólise (Bazotte *et al.* 1989; Beuers & Jungermann 1990; Doi *et al*. 2001; Ikeda *et al*. 1989; Vardanega-Peicher *et al.* 2003). A técnica de perfusão do fígado tem por objetivo estudar o metabolismo e foi realizada pela primeira vez entre 1902 e 1912 por Embden e seus colaboradores, utilizando fígados de cães e gatos. As primeiras perfusões de fígado de rato foram feitas por Corey e Britton em 1941 e por Trowell em 1942. Essa é uma técnica na qual os vasos aferentes e o vaso eferente do órgão são canulados de tal maneira que se pode controlar a qualidade do líquido arterial e coletar, para posterior análise, o líquido venoso, sendo para isso utilizadas bombas para impulsionar o líquido de perfusão através do fígado. No fígado em perfusão, podem ser medidas várias vias metabólicas, como a gliconeogênese, a glicólise, a glicogenólise, o consumo de oxigênio, a cetogênese, a captação de ácidos graxos, a frutólise, o metabolismo do glicerol e a biotransformação de drogas. Os parâmetros mais fáceis de serem medidos são a liberação de glicose, lactato, piruvato e o consumo de oxigênio. A glicose é liberada em sua maior parte porque a velocidade de síntese de glicogênio é muito menor que a produção de glicose. Lactato e piruvato são liberados em sua maior parte porque a velocidade de sua produção é superior à capacidade das células de oxidá-los na cadeia respiratória (Bracht *et al*. 2003).

A manutenção da homeostase da glicose é um processo altamente complexo, que requer o funcionamento adequado de múltiplas vias metabólicas e tipos celulares. Um ambiente nutricional pobre durante a gestação e lactação está relacionado ao aumento da suscetibilidade de desenvolver intolerância a glicose e diabetes mellitus tipo 2 na idade adulta (Hales & Barker 1992,

Poulsen *et al*. 1997). De acordo com essa hipótese, denominada “Hipótese do Fenótipo Econômico” uma condição nutricional desfavorável durante as fases críticas do desenvolvimento, promoveria uma “programação metabólica” que durante a vida fetal e neonatal garantiria a sobrevivência do feto, mas predisporia ao surgimento de doenças na maturidade, como a síndrome metabólica (Barker *et al*. 2002, Peer *et al*. 2014).

Esses efeitos são vistos em estudos epidemiológicos tais como o realizado com descendentes nascidos durante a fome Holandesa (1944-1945), onde indivíduos expostos a um ambiente nutricional pobre durante a vida fetal, apresentavam baixo peso ao nascer (Roseboom *et al.* 2001) e estavam mais propensos a desenvolver doenças na idade adulta tais como, resistência à insulina e diabetes tipo 2 (Painter *et al. 2006*).

Não se sabe qual tipo de deficiência nutricional torna o feto mais susceptível a desenvolver doenças na idade adulta, porém grande atenção é dada a restrição proteica, pois os aminoácidos são importantes para o crescimento fetal, desenvolvimento das células β pancreáticas e secreção de insulina (Hales & Barker, 1992). Estudos utilizando roedores submetidos à restrição proteica nas fases críticas do desenvolvimento, são amplamente utilizados como modelo animal de programação metabólica, para entender o impacto da má nutrição intrauterina e o surgimento de doenças metabólicas no adulto (Liu *et al*. 2014). Esses estudos mostraram que a prole de ratas mantidas com dieta hipoproteica durante a gestação e lactação, apresentaram número reduzido de células β pancreáticas (Berney *et al*. 1997), redução do tamanho e vascularização das ilhotas pancreáticas (Snoeck *et al*. 1990), redução da densidade, massa absoluta e conteúdo de insulina das células β (Garofano *et al*. 1997).

Além disso a restrição protéica altera a expressão de genes e a atividade de enzimas hepáticas envolvidas no metabolismo da glicose. No fígado, a atividade da glicoquinase está diminuída e a da fosfoenolpiruvato carboxiquinase (PEPCK) está aumentada e estas alterações são acompanhadas por mudanças paralelas nos níveis do RNA mensageiro dessas enzimas (Desai *et al*. 1995; Desai *et al*. 1997).

Intervenções dietéticas têm se mostrado úteis no tratamento e na prevenção de doenças crônicas, como o diabetes tipo 2, e o consumo de soja

(*Glycine max*) tem sido associada a esses benefícios (Bhathena & Velasquez 2002).

A soja é composta por aproximadamente 40% de proteína, 21% de lipídeos, 34% de carboidratos, 5% de cinzas, sendo que 8% do seu peso corresponde a casca (Morais & Silva 2000). Apresentando um grande potencial no mercado de alimentos funcionais, devido à presença de compostos bioativos, como as isoflavonas, as quais têm sido largamente estudadas quanto aos seus efeitos biológicos benéficos à saúde humana (Esteves & Monteiro 2001).

As isoflavonas estão presentes nos tecidos vegetais na forma inativa (genistina, daidzina e gliciteína) (Esteves & Monteiro 2001). Estas, uma vez ingeridas, são hidrolisadas no intestino por bactérias β-glicosidases sendo convertidas em suas formas "agliconas bioativas" (genisteína, daidzeína e gliciteína) (Anderson & Garner 1997). A genisteína (principal isoflavona da soja) afeta favoravelmente a homeostase da glicose, pois tem efeito sobre a secreção de insulina, absorção intestinal de glicose e metabolismo da glicose no fígado, adipócitos e músculo esquelético (Liu *et al*. 2006; Huppertz *et al*. 2001; Szkudelska *et al*. 2000). Em hepatócitos isolados, a genisteína ativa levemente a glicogênio fosforilase e inativa a glicogênio sintase, provavelmente por aumentar os níveis de AMPc (Keppens 1995).

O aumento da secreção de insulina tem sido atribuído à composição de aminoácidos da proteína isolada de soja, especialmente às altas concentrações de arginina, um potente estimulador da secreção de insulina (Fajans *et al*. 1997, Sanchez & Hubbard, 1991). A arginina exerce ação insulinotrópica por seu acúmulo no interior das células β, que provoca a despolarização da membrana e a entrada de cálcio via canais de Ca^{2+} voltagem-dependentes (Blachier *et al*. 1989).

Estudos realizados no nosso laboratório de pesquisa mostraram que ratos alimentados com dieta à base de farinha de soja (tanto os recuperados como os que eram alimentados com dieta normoproteica 17% de caseína durante a vida intrauterina e lactação e passaram a receber uma dieta com 17% de proteína à base de soja após o desmame apresentaram menor peso corporal aos 90 dias, quando comparados com seus controles alimentados com dieta normoproteica (17% de caseína) (Cheim *et al.* 2009, Reis *et al.* 2015, de

Arruda Oliveira *et al*. 2008, Milanski *et al*. 2009, Feres *et al*. 2010), assim como menor peso do fígado e gordura hepática (Milanski *et al*. 2009). A concentração sérica de insulina foi maior nos animais alimentados com dieta à base de soja (de Arruda Olivera *et al*. 2008, Reis *et al*. 2015, Feres *et al*. 2010), associada a maior HOMA-IR (Reis *et al*. 2015) e sinalização hepática da insulina reduzida, se considerarmos a menor expressão da proteína IR e IRS-1 (Feres *et al*. 2010).

Outros estudos mostram que ratos recuperados com dieta à base de farinha de soja apresentam baixo conteúdo de glicogênio hepático e baixo nível de glicose basal, sem apresentar alteração da gliconeogênese hepática (resultados não publicados Pachecco, 2012). Tais achados sugerem que a baixa glicemia basal seria resultado dos baixos estoques de glicogênio, devido ao efeito da genisteína que contribui para aumentar a glicogenólise. Portanto, nosso trabalho tem como finalidade verificar a ação da dieta à base de farinha de soja sobre a produção de glicose, glicólise e glicogenólise hepática por meio de estudos com perfusão hepática.

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3. OBJETIVOS

GERAL

Avaliar o papel do glucagon e do AMPc na produção de glicose, glicólise e glicogenólise no fígado de ratos alimentados com dieta à base de farinha de soja desde o desmame e aqueles expostos à restrição proteica na vida intrauterina e lactação e recuperados com dieta à base de farinha de soja.

ESPECÍFICOS

- Avaliar os efeitos da dieta à base de farinha de soja em ratos adultos recuperados da desnutrição sobre:

- ✓ O peso corporal;
- ✓ A ingestão alimentar;
- ✓ O peso absoluto e relativo do fígado, tecido adiposo epididimal, tecido adiposo retroperitoneal e tecido adiposo marrom;
- ✓ As concentrações séricas de ALT e AST;

- Avaliar o efeito doglucagon e do AMPc sobre a produção hepática de glicose, glicólise e glicogenólise através da técnica de perfusão *in situ*.

4. ARTIGO

cAMP increases glucose hepatic prodution and glycolisis without effecting glycogenolisis in rats fed soybean diet

Effect of soybean diet on cAMP action in the hepatic glucose production, glycolisis and glycogenolisis

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Keywords: glucose production, glycolysis, glycogenolisis, soybean, protein restriction, recovery, rats

ABSTRACT

Poor nutritional environment in early life is related to increased susceptibility to glucose intolerance or type 2 diabetes mellitus during adult life and soybean utilization is indicated as being useful to prevention of these disorders. Due to its recommendation, our studies are focused to the action of soybean as recovery method for animal model of protein restriction during early life. However, our results are conflicting with the vision of potential positive effect of the diet based soybean, since it resulted in reduced level of hepatic glycogen and increased serum ALT. Thus, considering these results we investigated if the diet and/or the nutritional state could affect the action of glucagon and cAMP on glucose production, glycolysis and glycogenolisis by the liver perfusion technique.Male offspring from mothers fed with 17% or 6% protein (casein) during pregnancy and lactation were maintained with 17% casein (CC and LC groups) or soybean (CS and LS groups) diet and with 6% casein (LL group) diet until 90 days of life.Liver of recovery groups (LC and LS rats) has responded more effectively to cAMP actionin compare to LL group, suggesting higher sensitivity to glicolitic agent.Through cAMP perfusion, soybean diet groups showed increased glucose production, higher glycolysis inhibitionwithout significative increase in glycogenolisis. Thus, we suggest that the glucose produced by liver of soybean groups is, at least in part, recycled to be used in the glycogen synthesis as a manner of ensure some glycogen content in relation to control diet groups.

INTRODUCTION

Blood glucose concentrations in normal healthy individuals are normally maintained at ~90 mg/dl and among the tissues contributing to the maintenance of normal of blood glucose levels are the liver, skeletal and cardiac muscle, fat and brain (Sharabi *et al*. 2015), being the liver the main responsible for maintaining glucose homeostasis for its ability to store glucose as glycogen (glycogenesis), producing glucose through the breakdown of glycogen (glycogenolysis) or de novo glucose synthesis from available precursors (gluconeogenesis) (Lin & Accili 2011, Roden & Bernroider 2003).

Insulin and glucagon are hormones that control the of utilization and production of glucose to maintain glycemia (Samuel & Shulman 2012). When there is an increase in blood glucose, insulin is secreted from pancreatic β cells and promotes hepatic glycogen synthesis and lipogenesis. On the other hand, when it decreases the blood glucose levels, glucagon is secreted from pancreatic α cells to promote hepatic glucose production (HGP) (Lin & Accili 2011).

The binding of glucagon to the extracellular loops of the glucagon receptor results in conformational changes, leading to subsequent activation of the coupled G proteins (Jiang & Zhang 2003). The activation of G proteins leads to activation of adenylate cyclase, increase in intracellular adenosine 3’5’-monophosphate cyclic (cAMP) levels, and subsequent activation of protein kinase A (PKA), which triggers a cascade of events that culminate in phosphorylation and activation of glycogen phosphorylase that phosphorylates the glycogen resulting in the glycogenolysis and the production of glucose-6-phosphate (G-6-*P*). G-6-*P* is then converted into glucose by glucose-6-phosphatase (G-6-Pase), increasing the hepatic production of glucose (Johnson *et al*. 1997, Krebs 1981).

The maintenance of glucose homeostasis is a complex process that requires the proper functioning of several metabolic pathways and cell types. A poor nutritional environment during pregnancy and lactation is related to increased susceptibility to glucose intolerance or type 2 diabetes mellitus during adult life (Hales & Barker 1992, Poulsen *et al*. 1997).

According to the theory proposed by Hales & Barker (1992), an unfavorable nutritional condition during the critical stages of development, promotes metabolic programming ensuring fetal survival during fetal and neonatal life, but would predispose to the emergence of diseases at maturity, such as the metabolic syndrome.

Perfusion study have demonstrated that glucagon stimulates glucose output from perfusion in rats livers resulting from increases in both glycogenolysis and gluconeogenesis and decrease in the glycolysis (Bazotte *et al.* 1989, Drouin *et al.* 2004, Mokuda *et al.* 1997, Vardanega-Peicher *et al.* 2003a).

Dietary interventions are usefulin the metabolic diseases and soybean utilization (*Glycine max*) has been described as an efficient measure for preventing and treatment of deseases at maturity.

Multiple studies in humans and animals suggest that isoflavones, such as the genistein found in soybean, are essential component in the action of reducing obesity and diabetes and improving glucose control and insulin resistance (Cederroth *et al*. 2008). Dietary genistein at doses of 500-1500 ppm had antilipogenic effects and reduced fat deposition (Naaz *et al*. 2003). Bu *et al*. 2005 and Lephart *et al*. 2004, showed that rodents fed with a soy-rich diet had significantly decreased adiposity compared with animals fed isoflavone-free diet.

As described, poor nutritional environment in early life is related to increased susceptibility to glucose intolerance or type 2 diabetes mellitus during adult life and soybean utilization has proved be useful to prevention of these alterations. Due to its recommendation, our studies are focused to the action of soybean as recovery method for animal model of protein restriction. However, our results are conflicting with the vision of potential positive effect of the diet based soybean, since it resulted in high serum AST and ALT and reduced level of liver glycogen stores (Reis *at al*. 2015).

Thus, considering that rats submetted to protein restriction during intrauterine life and recovery with soybean diet exhibited hepatic alteration of glycogen content, we investigated if the diet and/or the nutritional state could affect the action of glucagon and cAMP on glucose production, glycolysis and glycogenolysis by the liver perfusion technique.

MATERIALS AND METHODS

The study was conducted in the Laboratory of the Department of Food and Nutrition (LABA) of the Faculty of Nutrition at the Universidade Federal de Mato Grosso.

Animals and Diets

The experimental procedures involving rats were performed in accordance with the guidelines of the Brazilian College for Animal Experimentation (COBEA) and were approved by the ethics committee at the Federal University of Mato Grosso (Number Process 23108.061022/13-9). Male and virgin female Wistar rats (85–90 days old) were obtained from the university's own breeding colony. Mating was performed by housing males with females overnight (1 male and 4 females), and pregnancy was confirmed by the presence of sperm in vaginal smears. Pregnant females were separated at random and maintained from the first day of pregnancy until the end of lactation on isocaloric diets containing 6% protein (low protein - LP diet) or 17% protein (control -C diet). The offspring born spontaneously on day 21 of pregnancy, and after 3 days the brood was reduced to eight puppies to ensure a standard size of litter by mother. At 28 days of life the male offspring were divided into five groups: CC, consisting of offspring born to and suckled by mothers fed a C diet and subsequently fed the same diet after weaning until 90 days of age; CS, consisting of offspring born to and suckled by mothers fed a C diet and subsequently fed a soybean flour diet with 17% protein after weaning until 90 days of age; LL, consisting of offspring born to mothers fed an LP diet and subsequently fed the same diet after weaning until 90 days of age; LC, consisting of offspring born to mothers fed an LP diet and subsequently fed a C diet after weaning until 90 days of age; and LS, consisting of offspring born to mothers fed an LP diet and subsequently fed a soybean flour diet containing 17% protein after weaning until 90 days of age. The diets are presented in Table 1. In the soybean diet, adjustments were made to equalize the carbohydrate, lipids, fiber contents and energy value to a casein diet, suppressing the soybean oil and reducing fiber. Throughout the experimental period, the rats were given free access to food and water. They were kept under standard lighting conditions (12h light/ dark cycle) at a

temperature of 24°C. The rats were weighed at birth and at 30 and 90 days of life. The dietary intake was measured three times per week. All experimental procedures were performed at 90 days of age.

Table 1 – Composition of control, low-protein, and soybean flour diets*

Ingredient (g/Kg)	Control (17% protein)	Low protein (6% protein)	Soybean flour (17% protein)
Casein (84% protein)	202,0	71,5	-
Soybean flour	-	-	415,0
Cornstarch	397,0	480,0	312,2
Dextrinized cornstarch	130,5	159,0	103,7
Sucrose	100,0	121,0	78,6
Soybean oil	70,0	70,0	-
Fiber	50,0	50,0	40,0
Mineral mix (AIN-93)G*	35,0	35,0	35,0
Vitamin mix (AIN-93)G*	10,0	10,0	10,0
L-cystine	3,0	1,0	3,0
Choline bitartrate	2,5	2,5	2,5

* Reeves et al. 1993

Sample Collection and Analyses

At the end of the experimental period, at 90 days of age, the rats were decapitated and blood samples were collected and serum was obtained for subsequent determination of hepatic enzimes. The rats were then submitted to median laparotomy for removal of the liver, epididymal adipose tissue, retroperitoneal adipose tissue and brown adipose tissue.

Hepatic enzimes

Hepatic enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) were determined using the enzymatic method with a Labtest specific kit.

In situ liver perfusion

Anesthetized rats (ip injection of thiopental sodium, 40 mg/kg) were submitted to a laparotomy, and the livers were perfused in situ with a constant flux (4 $ml \cdot min^{-1} \cdot g^{-1}$). The weight of the liver was estimated as 4% of body weight. The perfusion buffer was pumped into a controlled temperature (37°C) membrane oxygenator prior to entering the liver via the portal vein. The collection of the effluent perfusion fluid was provided by a cannula inserted into the infrahepatic segment of the cava vein. The perfusion was performed in an open system without recirculation of the perfusate. Every animal was subjected to a preperfusion (20 min) with perfusion buffer (Krebs-Henseleit bicarbonate buffer without glucose saturated with O^2/CO^2 (95:5%), pH 7.4, during which samples of the effluent perfusion fluid were discarded. The composition of the KHB buffer was: 115 mM NaCl, 25 mM $NaHCO_3$, 5.8 mM KCl, 1.2 mM Na_2SO_4, 1.18 mM $MgCl_2$, 1.2 mM NaH_2PO_4 and 2.5 mM $CaCl_2$. Livers were perfused with KHB for the first 10 min, with KHB + cAMP (5 µM) between 10 and 60 min or with KHB for the first 10 min, with KHB + glucagon (1 nM) between 10 and 40 min. The effluent perfusate was collected every 2 min to measure the production of glucose, lactate and pyruvate. At the end of the perfusion, the liver was removed and weighed, to allow precise metabolic calculations and the correction of flux rates. Glycogenolysis was calculated as the sum of glucose production plus half the sum of lactate and pyruvate production [glucose + 1/2 (lactate + pyruvate)]. Glycolysis should be calculated by the sum of L-lactate plus pyruvate. The areas above curves of glucose production and glycogenolysis, and areas under curves of glycolysis were calculated for the interval of 10-60 min for infusion cAMP or 10-30 min for infusion glucagon.

Analytical procedures

The concentrations of glucose (Bergmeyer *et al*. 1974), pyruvate (Czok *et al*. 1974) and lactate (Gutmann *et al*. 1974) were assayed by enzymatic methods.

Statistical Analysis

The results are expressed as the means with their respective standard deviations for the number of rats indicated in parentheses. Levene's test for the

homogeneity of variances was initially used to determine whether the data complied with the assumptions necessary for a parametric ANOVA. When necessary, the data were log transformed to correct for variance in heterogeneity or nonnormality (Sokal & Rohlf 1995). A two-way ANOVA (i.e., effects of nutritional status in early life and diet) was used to compare the data from the CC, CS, LC, and LS groups. A one-way ANOVA was used to assess whether the diets were effective at improving the nutritional status of the LC, LS, and LL groups. When necessary, these analyses were complemented by the least significant difference test to determine the significance of the individual differences. $P< 0.05$ indicated statistical significance. All statistical comparisons were conducted using the Statistica software package (StatSoft). For other analysis the results are expressed as the means of the areas above curves with their respective standard deviations for the number of rats indicated in parentheses. To analyse data, a two-way ANOVA (i.e., effects of nutritional status in early life and diet) was used to compare the mean of the areas above or under curves and their confidence intervals from the CC, CS, LC, and LS groups. A one-way ANOVA was used to assess whether the diets were effective at improving the nutritional status of the LC, LS, and LL groups. $P< 0.05$ indicated statistical significance.

RESULTS

Protein restriction during pregnancy produced a significant reduction in neonatal body weight ($P<0.001$) (Table 2). At weaning, the mean body weight of the offspring from dams maintained on an low protein diet during pregnancy and lactation was significantly lower compared with that of offspring from dams maintained on a normal-protein diet in the same period ($P<0.001$) (Table 2).

During the recovery phase, the food intake was found to be lower in the LC and LS compared to CC and CS groups ($P<0.001$). CS and LS groups also showed lower food intake than the CC and LC ($P< 0.05$). When assessing nutritional recovery the food intake was similar between the LS and LC and significantly higher than the LL rats (Table 2).

According to table 2, body weight at the end of the experimental period was significantly lower in the LC and LS groups than in the CC and CS groups

(*P*<0.001). Rats maintained on a soybean flour diet after weaning (LS and CS groups) had a lower final body weight than those fed a casein diet (LC and CC groups) (*P*< 0.001). Although LS rats reached a higher final body weight than LL rats (*P*< 0.001), their weights were still significantly lower than those of LC rats (*P*< 0.001).When expressed per gram of body weight, the food intake was higher in rats maintained on a soybean flour diet (CS and LS groups), compared to CC and LC rats (P< 0.001). The food intake/100g body weight was higher in the LS than in LC group (P< 0.05), in compare to the LL group (*P*< 0.001).

Table 2–Body weight at birth, weaning and final, food intake, serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) from adult rats maintained on control (CC and LC groups) soybean flour (CS and LS groups), or low protein (LL group) diet after weaning until 90 days age.

	Groups				
Variables	**CC**	**CS**	**LC**	**LS**	**LL**
Body weight birth (g)	5.62 ±0.34 (12)	-	-	-	5.11 ±0.31* (12)
Body weight Weaning(g)	72.14 ±2.93 (12)	-	-	-	31.05 ±1.96* (12)
Food intake (g)	1056 ±82 (8)	994 ±53 # (8)	840 ±51*a (8)	768 ±70 *#a (8)	552 ±93 b (8)
Food intake (g/100 g BW)	227.1 ±21.8 (8)	255.2 ±18.0 # (8)	238.9 ±18.1 c (8)	271.4 ±27.6 #b (8)	467.8 ±41.43a (8)
Body weight final (g)	468 ±25 (12)	394 ±19 # (12)	357 ±21* a (12)	280 ±11* # b (12)	118 ±12 c (12)
ALT (U/L)	45.39 ±14.30 (4)	32.01 ±5.75 (4)	38.77 ±4.81 a (4)	37.59 ±7.34 a (4)	19.21 ±5.85 b (4)
AST (U/L)	54.36 ±16.51 (4)	44.30 ±9.92 (4)	51.94 ±5.49 (4)	51.29 ±12.56 (4)	49.65 ±17.54 (4)

The values are the mean ± SD of the number of rats indicated in parentheses. *Mean values were significantly different from the control rats (*p*< 0.05; two way ANOVA). #Mean values were significantly different from the rats fed a casein diet (*p*<0.05, two-way ANOVA). Means with superscript minuscule letters are significantly different by one-way ANOVA followed by LSD test (*p*< 0.05).

In the fed state, the serum concentration of AST was not significantly influenced by the pre-nutritional status, the diet used after weaning, as well as the interaction between these factors. The concentration of ALT was significantly influenced by recovery, thus it was found that LC and LS groups showed similar and significantly higher ALT serum concentrations than LL rats (P< 0.05) (Table 2).

Table 3 - Absolute and relative weight of white adipose tissue retroperitoneal (RET), epididymal (EPI), brown and liver from adult rats maintained on control (CC and LC groups) soybean flour (CS and LS groups), or low protein (LL group) diet after weaning until 90 days age.

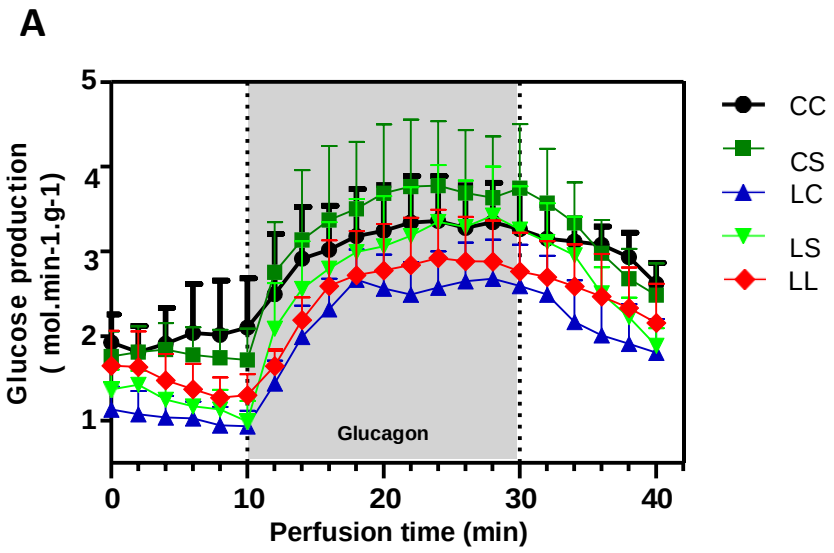
	Grupos				
Variáveis	**CC (6)**	**CS (6)**	**LC (6)**	**LS (5)**	**LL (6)**
			g		
RET	13.76 ±0.47 (6)	14.00 ±0.80 (6)	6.60 ±0.54* [a] (6)	5.87 ±0.82 * [b] (6)	4.38 ±0.40 [c] (5)
EPI	3.94 ± 0.48 (6)	4.49 ±0.48 [#] (6)	3.10 ±0.24 *[b] (6)	3.71 ±0.66 *[#a] (6)	1.59 ±0.37 [c] (5)
BROWN	0.24 ±0.01 (6)	0.29 ±0.01 [#] (6)	0.21 ±0.02 * [b] (6)	0.26 ±0.03 *[#a] (6)	0.24 ±0.01 (5)
LIVER	18.50 ±1.73 (6)	18.70 ±2.35 (6)	14.84 ±1.44* [a] (6)	15.44 ±2.34* [a] (6)	9.92 ±1.82 [b] (5)
			g/100g		
RET	2.76 ±0.18 (6)	2.73 ±0.12 [#] (6)	1.70 ±0.11 *[b] (6)	1.34 ±0.12 *[#c] (6)	2.13 ±0.21 [a] (5)
EPI	0.79 ±0.11 (6)	0.88 ±0.05 (6)	0.79±0.07 (6)	0.84 ±0.10 (6)	0.76 ±0.08 (5)
BROWN	0.05 ±0.006 (6)	0.06 ±0.005 [#] (6)	0.05±0.005 [b] (6)	0.06 ±0.008 [#b] (6)	0.12 ±0.014 [a] (5)
LIVER	3.74 ±0.28 (6)	3.64 ±0.29 (6)	3.80 ±0.23[b] (6)	3.56 ±0.50[b] (6)	4.79 ±0.67 [a] (5)

The values are the mean ± SD of the number of rats indicated in parentheses. *Mean values were significantly different from the control rats (p< 0.05; two way ANOVA). [#]Mean values were significantly different from the rats fed a casein diet (p<0.05, two-way ANOVA). Means with superscript minuscule letters are significantly different by one-way ANOVA followed by LSD test (p< 0.05).

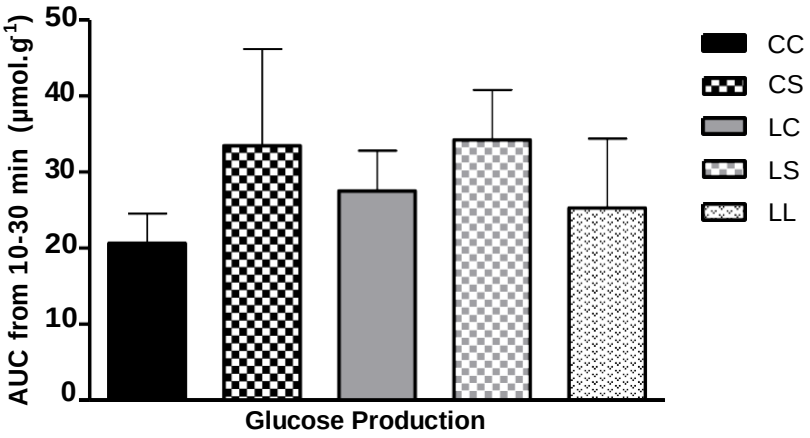
Epididymal brown and retroperitoneal adipose tissue was significantly lower in the LC and LS groups than in the CC and CS groups (P<0.01). Althought the

LS rats had a higher retroperitoneal adipose tissue than the LL rats their weight were still significantly lower than LC rats. CS and LS groups also showed lower weight of epididymal (P< 0.05) an brown (P< 0.001) adipose tissues than the CC and LC groups. LS group was higher weight epididymal (P< 0.05) and brown (P< 0.05) adipose tissue than LC group. These groups were higher than LL group when observed the epididymal adipose tissue (P< 0.001), but when observed brown adipose tissue, LC and LS groups did not have significant difference when compared to the LL group. The liver weight was found to be lower in the LC and LS compared to CC and CS groups (*P*< 0.001). When assessing nutritional recovery the liver weight was similar between the LS and LC and significantly higher than the LL rats (P< 0.001) (Table 3).

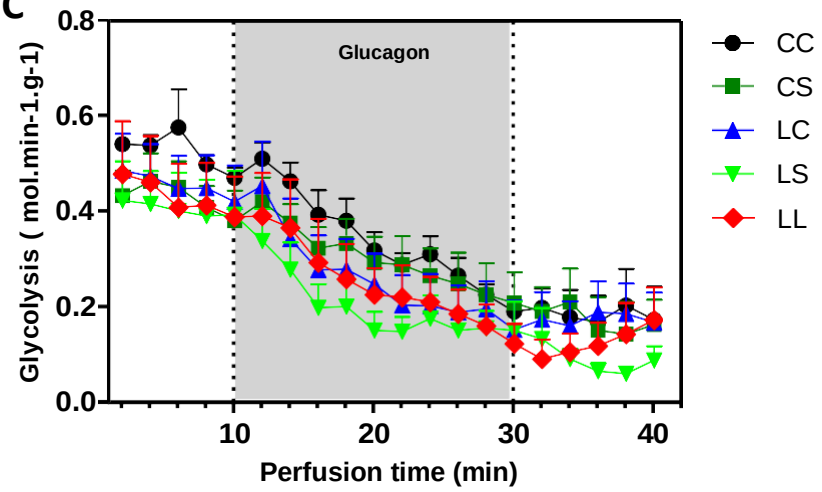
The weight of retroperitoneal and brown adipose tissue when expressed per 100g of body weight were significantly affected by the diet used after weaning, so the weight of these tissues in animals fed soy diet after weaning was significantly lower than in animals fed casein diet. Retroperitoneal adipose tissue was significantly lower in the LC and LS groups than in the LL group (*P*< 0.05).When evaluating nutritional recovery, it was verified that the LC and LS groups had similar and significantly lower brown adipose tissue (P< 0.001) and liver (P< 0.05) weight than the LL group.On the other hand, the percent contribution of the liver to the body weight was the same among the CC, CS, LS and LC groups. The epididymal adipose tissue did not differ between the three groups (Table 3).



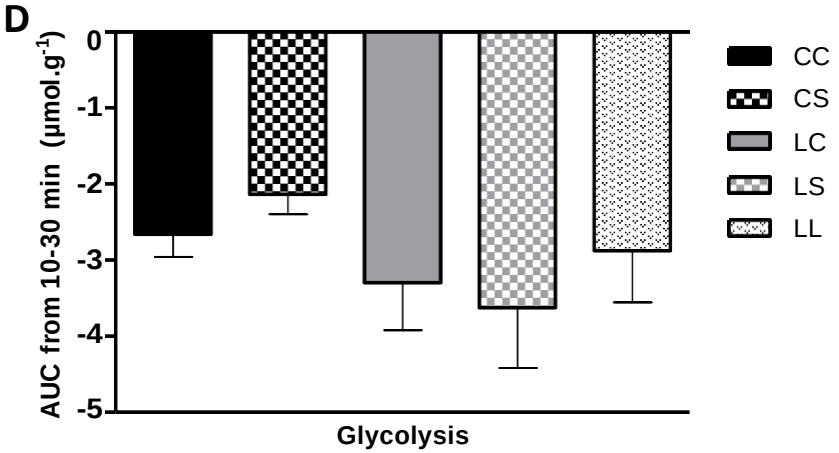
B


AUC from 10-30 min (µmol.g-1)
50
40
30
20
10
0
Glucose Production
CC
CS
LC
LS
LL

C


Glycolysis (mol.min-1.g-1)
0.8
0.6
0.4
0.2
0.0
Glucagon
10
20
30
40
Perfusion time (min)
CC
CS
LC
LS
LL

D


AUC from 10-30 min (µmol.g-1)
0
-1
-2
-3
-4
-5
Glycolysis
CC
CS
LC
LS
LL

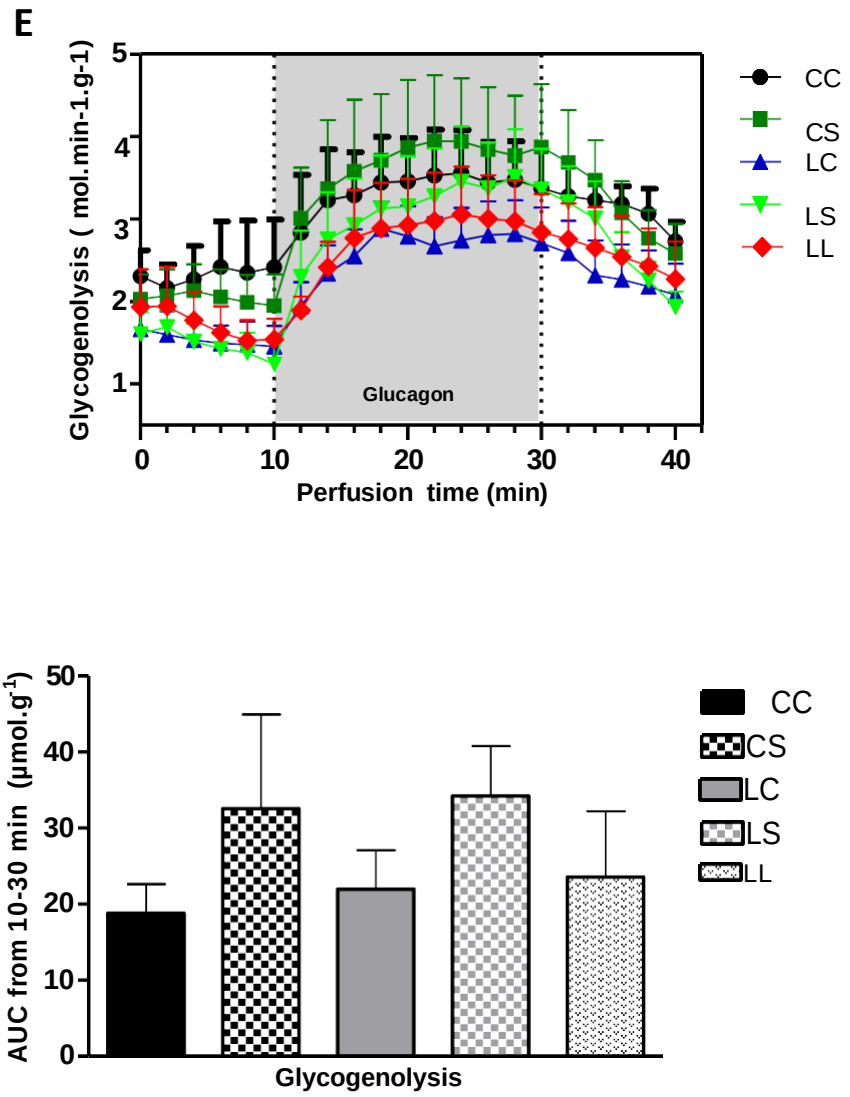


Figure 1 – Glucose production (A), glycolysis (C) and glycogenolysis (E) stimulated by glucagon 1 nM and the corresponding areas under curves (AUCs) (B, D, E) in livers of adult rats maintained on casein control (CC and LC), soybean flour (CS, LS) or low-protein (LL) diets after weaning. The livers were perfused as described in materials and methods. The values are expressed as the mean ± SD (n=4-6 rats). *Mean values were significantly different from the control rats (p<0.05; two way ANOVA). #Mean values were significantly different from the rats fed a casein diet (p<0.05, two-way ANOVA). Means with superscript minuscule letters are significantly different by one-way ANOVA followed by LSD test (p< 0.05).

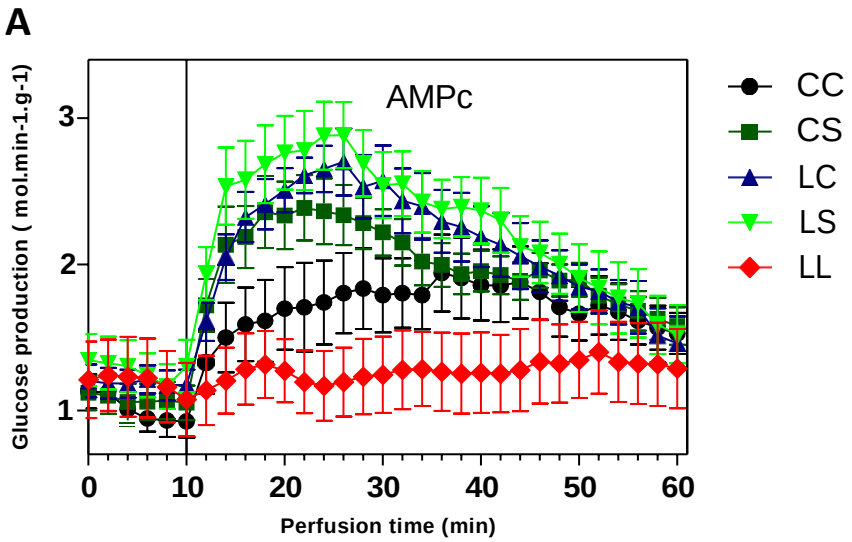
The hepatic response to glucagon (1 nM) in the production of glucose, glycolysis and glycogenolysis evaluated in perfusion in liver are shown in Figure 1A, B, C, D, E and F.

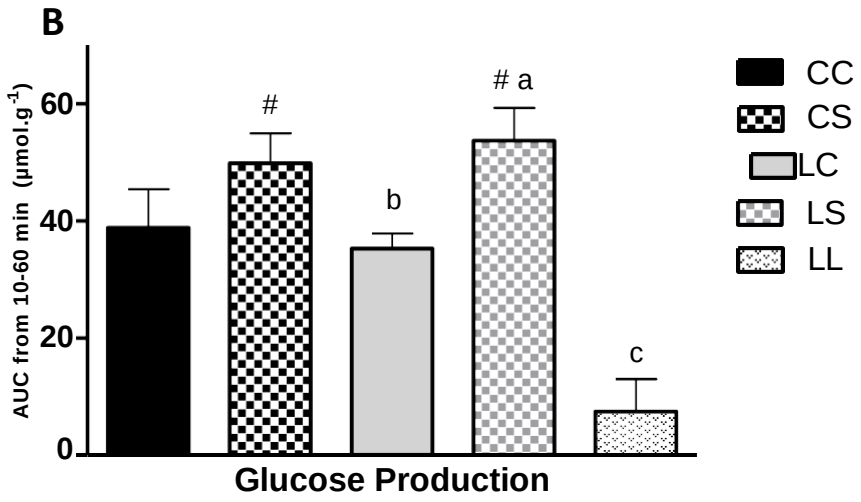
Glucagon infusion from 10 to 30 minutes did not promote significant change in both rats fed control diet (CC and LC), and in both rats fed soybean flour diet

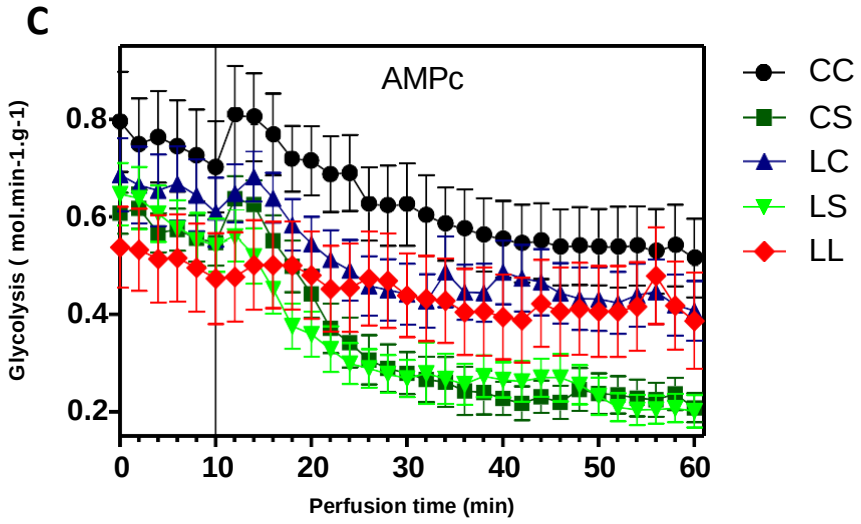
(CS and LS) and in low-protein diet (LL), as can be observed by the areas under the curve (AUC) (Figures 1B, 1D, 1F).

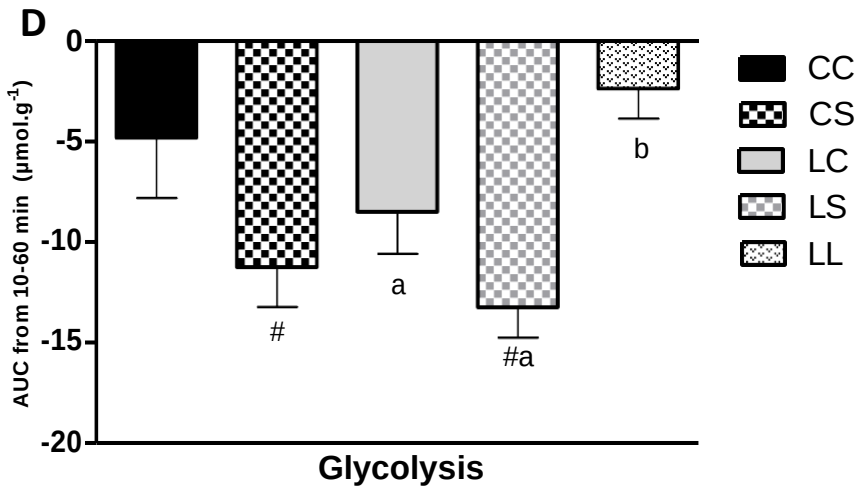
The hepatic response to 5 μM cAMP on the production of glucose, glycolysis and glycogenolysis evaluated in perfusion in rat liver of rats fed with control diet (CC and LC), soybean flour (CS and LS) and low-protein diet (LL) are shown in Figure 2.

Hepatic infusion of cAMP from 10-60 minutes of perfusion increased hepatic glucose production in CS and LS animals when compared to CC and LC animals($P< 0.05$).The recovered groups (LC and LS) presented higher hepatic glucose production in relation to theLL group($P< 0.001$).However, the animals recovered with soybean (LS) showed higher hepatic glucose production in relation to the group recovered with casein ($P <0.05$) and this was higher than the LL group ($P <0.001$) (Figure 2A, B) .




B
AUC from 10-60 min (µmol.g-1)
60
40
20
0
#
b
a
c
Glucose Production
CC
CS
LC
LS
LL


C
AMPc
Glycolysis (mol.min-1.g-1)
0.8
0.6
0.4
0.2
0
10
20
30
40
50
60
Perfusion time (min)
CC
CS
LC
LS
LL


D
AUC from 10-60 min (µmol.g-1)
0
-5
-10
-15
-20
#
a
#a
b
Glycolysis
CC
CS
LC
LS
LL

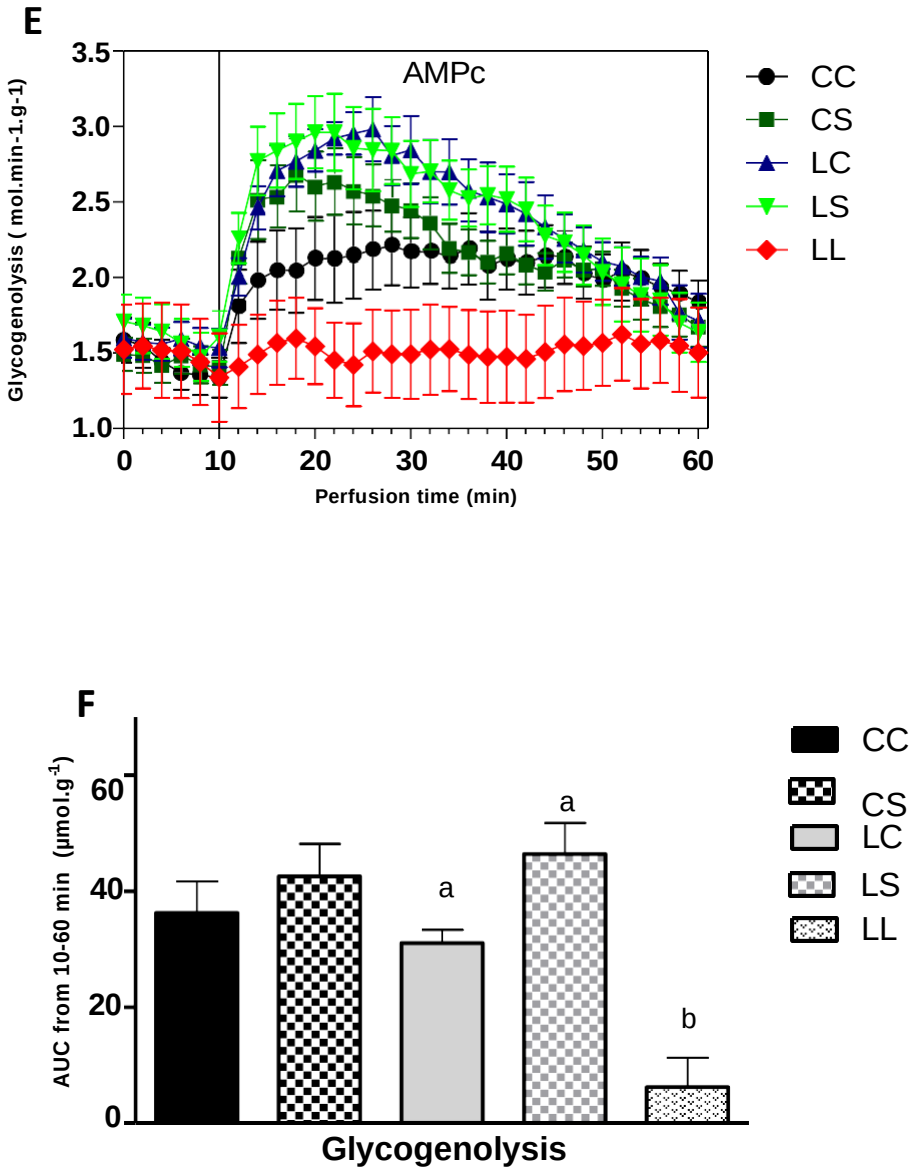


Figure 2 – Glucose production (A), glycolysis (C) and glycogenolysis (E) stimulated by cAMP 5µM and the corresponding areas under curves (AUCs) (B, D, E) in livers of adult rats maintained on casein control (CC and LC), soybean flour (CS, LS) or low-protein (LL) diets after weaning. The livers were perfused as described in materials and methods. The values are expressed as the mean ± SD (n=8-10 rats). *Mean values were significantly different from the control rats (p<0.05; two way ANOVA). #Mean values were significantly different from the rats fed a casein diet (p<0.05, two-way ANOVA). Means with superscript minuscule letters are significantly different by one-way ANOVA followed by LSD test (p< 0.05).

When glycolysis was evaluated, the animals fed soybean diet (CS and LS) had lower glycolysis when compared to the animals fed casein diet (CC and LC) (P <0.05).The recovered groups (LC and LS) had lower glycolysis than the

hypoproteic group (LL) ($P < 0.05$), and there was no significant difference between groups recovered (Figure 2C, D).

Glycogenolysis in the groups fed with casein diet (CC and LC) and soybean diet (CS and LS) had no significant difference after hepatic infusion of cAMP. The recovered groups (LC and LS) had higher glycogenolysis when compared to the hypoproteic group (LL) ($P < 0.001$) (Figure 2 E, F).

DISCUSSION

In the present study, we investigated the effect of soybean diet offered as recovery after weaning to rats submitted to protein restriciton during early life (LS group) and to control rats fed such a diet after weaning (CS group), on glucose poduction, glycolisis and glycogenolisis. As described previously (Latorraca *et al*. 1998), the low protein from early to adult life resulted in rats with low weight in all life stages. In addition, the nutritional recovery after weaning did not correct the weight body, retroperitoneal and brown adipose tissue and liver weights in compare to LL group, independently of protein source be animal or plant (Shepherd *et al*. 1997; Cheim *et al*. 2009). Similar glucagon effects were shown by the liver of recovery (LC and LS) and LL groups, while recovery groups has responded more effectively to cAMP action, suggesting higher sensitivity to glycolitic agent.

β-conglycinin, a soy protein, and fragments of soy peptides rich in arginine residues show high food intake suppressor activity and potentially delay gastric emptying by increasing cholecystokinin levels in rats (Torres *et al*. 2006). From these mechanisms the expectancy of soybean to lead to lower body weight is created, however our soybean groups showed lower body weight and increased relative food intake. Such an opposite effec tcould be related to, at least, the presence of antinutritional factors in the soybean, which impair the protein digestibility and bioavailability affecting negatively the body weight (Cheim *et al*. 2009).

Soy protein consumption reduces hepatic lipotoxicity by maintaining adipocytes functionality, besides it reduces the lipogenic rate (Tovar *et al*. 2005). Soybean diet has been associated to reduction of fat storage due to the isoflavone action that increase energy expenditure by altering the brown adipose tissue activity

(Lephart *et al*. 2004). In the present study, despite increased brown adipose tissue weight, we suggest that similar to shown by Paiva *et al*. 2012, that investigated the same animal model, the thermogenic capacity of the brown adipose tissue was not affected by the soyabean diet.

In the present study, soybean diet was effective in the reduction of retroperitoneal tissue and also probabily to reduction of lypolisis stimulated by isoproterenol, as observed by Paiva *et al*. 2012. Lipolytic flux tightly correlates with hepatic glucose production through two mechanisms (Mittelman and Bergman, 2000; Rebrin *et al*. 1996): 1) by providing the liver with the gluconeogenic precursor glycerol and 2) by enabling the synthesis of high energy NADH substrates required during gluconeogenesis through β–oxidation of NEFAs (Hers and Hue, 1983). In addition, about two thirds of the hepatic glycogen originate from de novo formed glucose (Kuwajima et al. 1986). Despite gluconeogenesis and glycogenesis were not our goal, its relation to lypolisis and to glycogen synthesis could clarify the mechanism envolved in reduced glycogen content showed by soybean diet groups. In this context, we suggest that the poor contribution of gluconeogenic components could be involved in the reduced glycogen hepatic content observed in CS and LS groups.

Glucagon, the counter-regulatory hormone to insulin in control of glycemia and inductor of hepatic glycogenolysis, has its secretion enhanced by soy protein long-term intake, despite its concentration depends on the amount and composition of the ingested protein (Torres *et al*. 2006). In previous studies of our group, however, no significant change in glucagon serum was observed and the glucagon sensitivity in LS rats was reduced, indicating glucagon resistance (unpublished results Pacheco *et al.* 2012). In glucagon resistance state in hepatocites, the activation of adenylate cyclase that promotes the conversion of ATP to cAMP can be reduced avoiding the activation of glucose hepatic production. Perfused liver with the glucagon 1 nM showed no significant difference for glycolysis, glucose production and glycogenolysis among the groups, suggesting that the signaling pathway of the liver to mobilize glycogen was maintained in control and soybean groups.A possibility to this result may be that other counter-regulatory hormone, such a catecholamine could contribute to breakdown of glycogen in soybean diet groups (Fedatto-Júnior *et al*. 2002).

Due to confliting data in relation to glucagon perfusion test, we decided to use as glycolitic agent the second messenger cAMP. Soybean diet groups showed increased glucose production perhaps by overcoming cAMP production stimulated by glucagon 1nM in the previous test. In addition, soybean diet groups have showed higher glycolysis inhibition when compare to diet casein groups, indicating that soybean becames the liver more sensitive to such action. No reduction ofprotein expression of the glycolysis enzymes are attributed to the soybean components, while the activity, at least of phosphofrutokinase (PFK), could be indirectly inhibited by PKA, since it reduces the fructose 2,6-biphosphate level, a potent activator of PFK (Pilkis, El-Maghrabi & Claus 1990). In addition, genistein has a positive effect on cAMP levels in rat hepatocytes (Keppens, 1995).

Genistein is also able to inactivate glycogen synthase and slows down the glucose-induced inactivation of glycogen phosphorylase (Keppens, 1995). In opposite to described by Keppens (1995), an increase of glycogenolysis rate was not induced by soybean diet, despite higher glucose production. Glycogenolysis in perfused liver experiments is calculated as the sum of glucose production plus half the sum of lactate and pyruvate production (Vandanega-Peicher *et al*. 2003). If taking in account the high glucose production by soybean groups and its reduced pyruvate and lactate production (reduced glycolisis) the later could have been the determinant component to prevent the expected increase in glycogenolysis in LS and CS groups.

The hypothesis of our study was that the lower glycogen hepatic content described previously (de Arruda Oliveira *et al.* 2008, Cheim *et al*. 2009, Reis *et al.* 2015), would be due to increased glycogenolysis provided by soybean components, mainly genistein, which increase the cAMP and consequently, the PKA activity, besides its action on key enzymes involved in glycogen metabolism (Keppens, 1995). Here, it was evident that livers from soybean diet groups have a high capacity of releasing glucose derived from endogenous glycogen when stimulated by cAMP; of increasing the inhibition of glycolysis, without significative increase in glycogenolysis.Thus, we suggest that the glucose produced by liver of soybean groups is, at least in part, recycled to be used in the glycogen synthesis as an way of ensure glycogen content, even low in relation to control diet groups.

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