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"INFLUÊNCIA DA HIPERGLICEMIA E DO ESTRESSE OXIDATIVO NA CINÉTICA DE PROLIFERAÇÃO E MORTE CELULAR NO EPITÉLIO ACINAR DA PRÓSTATA DE RATOS DIABÉTICOS"

Dissertação apresentada ao Instituto de Biologia para obtenção do Título de Mestre em Biologia Celular e Estrutural, na área de Biologia Celular.

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Sumário

Lista de abreviaturas

AGE - produtos finais de glicação não-enzimática
AKT - quinase de serina-treonina
AOS - sistema de defesa antioxidante
AR - receptores de andrógenos
BBDP/Wor - *Diabetes prone bio breeding*
C - controle
CAT - catalase
CEL - Nε-(carboxietil) lisina
CgA - cromogranina A
CML - Nε-(carboximetil) lisina
CDNB - 1-chloro-2,4-dinitrobenzeno
CT - controles com glicemia ≤110 mg/dL
C + V - controle tratado com vitamina C
DHT - dihidrotestosterona
DM1 - *diabetes mellitus* tipo 1
DM2 - *diabetes mellitus* tipo 2
D - diabético
D + V - diabético tratado com vitamina C
GPx - glutationa peroxidase
GR - glutationa redutase
GSH - glutationa reduzida
GSSG - glutationa oxidada
GST - glutationa s-transferases
IDE - diabéticos tratados com insulina com glicemia normal abaixo de ≤130 mg/dL
IDH - diabéticos tratados com insulina com glicemia entre 250 – 450 mg/dL
IGF - fator de crescimento semelhante à insulina
IR - receptor de insulina
K - queratinas
LHRH - hormônio de liberação do hormônio luteinizante
MAPK - proteína quinase ativada por mitógeno
MDA - malondialdeído
mTOR - *mammalian target of rapamycin*
NFκB - fator de transcrição kappa B
NOD - *non obese diabetic*
PI3K - fosfatidil inositol 3-quinase
PCNA - antígeno nuclear de proliferação
PIA - atrofia prostática proliferativa
PIN - neoplasia intraepitelial prostática
PSA - antígeno específico prostático
RAGE - receptores de AGE
ROS - espécies reativas de oxigênio
SHBG - proteínas séricas de ligação dos hormônios sexuais

SMC - células musculares lisas
SOD - superóxido dismutase
STZ-D - diabetes induzido por estreptozotocina
TAC - *transit-amplifying cells*
TGF-ß - fator de transformação do crescimento beta
T/E - razão testosterona/estradiol
UD1 - diabéticos sem tratamento com glicemia entre 250 – 450 mg/dL
UD2 - diabéticos sem tratamento com glicemia acima de 550 mg/dL
UGS - seio urogenital endodermal
VP – próstata ventral de rato

Resumo

Os prejuízos do diabetes sobre a morfofisiologia prostática são conhecidos e associados à falta de insulina, queda de andrógenos e hiperglicemia. Estudos anteriores com diabetes mostraram ampla variação individual da resposta histológica da próstata frente a esta doença. Visando esclarecer os fatores responsáveis por essa variação, foram examinadas as correlações entre resposta histológica da próstata com a glicemia, com os níveis séricos de testosterona e estrógeno no diabetes em curto termo. Sabe-se que a hiperglicemia leva à formação de produtos finais de glicação não-enzimática (AGE) e à conseqüente superprodução de espécies reativas de oxigênio. Um aumento na apoptose foi constatado previamente no epitélio prostático após longos períodos de diabetes e da queda androgênica, sugerindo a influência da hiperglicemia e do estresse oxidativo nesse processo. Assim, no primeiro experimento, foi analisado o impacto do diabetes induzido por estreptozotocina no sistema antioxidante (AOS) da próstata ventral de ratos (VP) e a influência da suplementação com vitamina C (ácido ascórbico). Também foram examinadas as repercussões do estresse oxidativo na sensibilidade androgênica e cinética de proliferação e morte celular dessa glândula. Para isso, induziu-se o diabetes em ratos Wistar adultos pela estreptozotocina (4 mg/100g peso corporal), seguidos ou não do tratamento com vitamina C (150 mg/kg peso corporal/dia), via gavagem. Foram formados os seguintes grupos: controle (C), controle tratado com vitamina C (C+V), diabético (D) e diabético tratado com vitamina C (D+V). Os animais foram sacrificados após 30 dias de diabetes e a VP foi processada. Os níveis de malondialdeído (MDA) e as atividades de catalase (CAT), superoxido dismutase (SOD), glutationa peroxidadase (GPx) e glutationa S-transferase (GST) foram mensurados na próstata e no sangue. A vitamina C diminuiu os níveis de apoptose elevados pelo diabetes, porém não normalizou a proliferação celular nem protegeu contra o dano oxidativo. O AOS sangüíneo não foi afetado com um mês de diabetes, porém os níveis de CAT e GST aumentaram na glândula. O MDA e a expressão de Nε-(carboxymetil) lisina (CML), um dos principais AGE, também elevaram marginalmente nos grupos diabéticos. Também foi realizado um segundo experimento de indução do diabetes pela aloxana (42 mg/kg de peso corpóreo) com ou sem tratamento com insulina (5 UI/dia) As alterações morfológicas na VP foram examinadas em cortes histológicos em historresina, segundo as diferentes faixas de glicemia. Constatou-se uma relação inversa entre

a glicemia e a razão testosterona/estrógeno com o peso da VP. A atrofia nas extremidades distais dos ductos mostrou-se associada com altos valores glicêmicos. A insulina não impediu as alterações causadas pelo diabetes, principalmente quando o controle da glicemia não foi eficaz. Deste modo, o dano oxidativo é responsável, parcialmente, pelo desequilíbrio na proliferação e morte celular causados pelo diabetes. A GST é um bom indicador da defesa antioxidante na próstata nos estágios iniciais dessa desordem metabólica e o aumento de sua atividade pode estar relacionado com o subseqüente desenvolvimento de lesões malignas. Nossos dados indicam que a hiperglicemia prolongada aliada ao desequilíbrio hormonal sejam os principais responsáveis pelas alterações drásticas na VP dos animais diabéticos sem reposição de insulina.

Abstract

The diabetes damages in prostate morphophysiology are well known and are triggered by insulin lack, low androgen levels and hyperglycemia. Previous researches showed an individual variation in morphological prostate response against this disease. In order to clarify the factors responsible for this variation, we examined the correlation between histological response of the prostate with glycemia, serum testosterone and estrogen levels under short term diabetes. It is known that high glucose levels lead to the formation of end products of non-enzymatic glycation (AGE) and the subsequent overproduction of reactive oxygen species. An apoptosis increase was also reported in prostatic epithelium after long periods of diabetes and androgen drift, suggesting the influence of hyperglycemia and oxidative stress in this imbalance. The first experiment was performed to evaluate the impact of estreptozotocin-induced diabetes in the antioxidant system (AOS) of rat ventral prostate (VP) and the influence of supplementation with vitamin C (ascorbic acid). The effects of oxidative stress in androgen sensitivity and proliferation and cell death kinetics, in this gland, were also assessed. For this purpose, diabetes was induced in adult male rats by streptozotocin (4 mg/100g b.w) followed or not by treatment with vitamin C (150 mg/kg b.w./day), by gavage. The following groups were formed: control (C), control treated with vitamin C (C+V), diabetic (D) and diabetic treated with vitamin C (D+V). The animals were sacrificed after 30 days of diabetes onset and the VP was processed. The malondialdehyde levels (MDA) and catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione S-transferase (GST) activities were measured in the prostate and blood. Vitamin C reduced the high apoptosis levels due to diabetes, but did not normalize cell proliferation and it was not totally efficient against oxidative damage. The blood AOS was unaffected by one month of diabetes, but the CAT and GST activities were increased in the gland. The MDA levels and Nε-(carboxymetil) lysine (CML) expression, one of the main AGE, were marginally increased in diabetic groups. A second experiment of diabetes induction was based in alloxana induction (42 mg/kg b.w.) with or without insulin treatment. Morphological changes in the VP were examined in histological hystoresin sections, according to different ranges of glycemia. Glucose levels and testosterone/estrogen ratio were inversely related to the VP weight. The atrophy in the distal ends of prostate ducts was associated with high blood glucose levels. Insulin does not prevent the

changes caused by diabetes, especially if glycemic control is not effective. These results suggest that oxidative damage is partly responsible for the imbalance in proliferation and cell death caused by diabetes. GST is a good indicator of prostate antioxidant defense in the early stages of this metabolic disturbance and its increased activity may be related to the malignant lesions establishment. Our data indicates that prolonged hyperglycemia combined with hormonal disequilibrium are the main responsible for the drastic changes in the VP of diabetic animals without insulin replacement.

Introdução

1. Generalidades sobre o diabetes

O *diabetes mellitus* é um conjunto de desordens metabólicas, de origem genética ou ambiental, caracterizado pela hiperglicemia resultante de defeitos na secreção e/ou ação da insulina. Entre os sintomas característicos do diabetes destacam-se a poliúria (alto volume de urina), a polidipsia (sede), a cetoacidose e a perda de peso. Estes distúrbios acarretam inúmeras complicações sistêmicas envolvendo principalmente o sistema cardiovascular, excretor, nervoso e ocular podendo levar ao severo comprometimento na qualidade de vida dos indivíduos (Stefan, 1996; Avedano *et al*., 1999; Sanai *et al*., 2000; Lamers *et al.*, 2007). Atualmente, afeta 246 milhões de pessoas ao redor do mundo, e estima-se que afetará cerca de 380 milhões em 2025 (Ziaei-Rad, 2010). Desse modo, o diabetes constitui um grave problema de saúde mundial.

Dois quadros clínicos são classicamente descritos de acordo com a etiologia: o *diabetes mellitus* tipo 1 (DM1), caracterizado por uma total deficiência de insulina e o *diabetes mellitus* tipo 2 (DM2), que se instala por uma ação ineficiente da insulina nos tecidos periféricos (Öztürk *et al.,* 1996). Muitos indivíduos portadores de DM1 eventualmente se tornam dependentes de insulina para a sua sobrevivência (Ordóñez *et al.,* 2007). Por sua vez, o DM2, está relacionado à obesidade e ao sedentarismo que leva a situação de resistência periférica à insulina, culminando com essa desordem metabólica (Suthagar *et al.*, 2008). No mesmo âmbito de definição de diabetes pertencem ainda o diabetes gestacional e outras alterações de origens diversas no metabolismo e homeostase da glicose, como a resistência periférica à insulina (McCance *et al.,* 1997; Schinner *et al.,* 2005.)

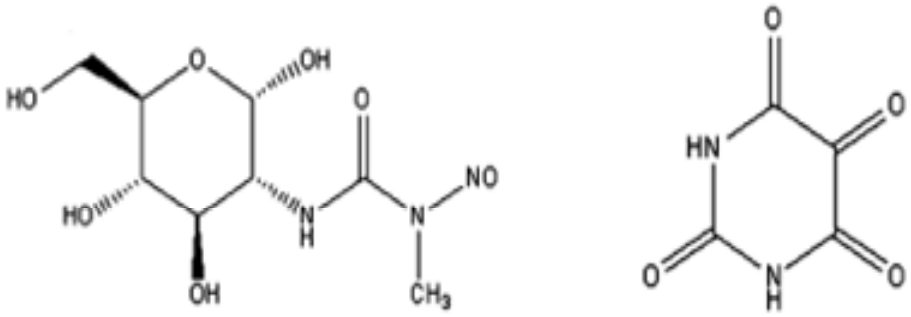
A ausência de insulina no *diabetes mellitus* do tipo 1 é devida à destruição autoimune das células β pancreáticas. Essa patologia autoimune é caracterizada pela presença de linfócitos T auto-reativos e anticorpos reativos contra a estrutura das células β. A incidência na população do DM1 é de aproximadamente 25/100 000 com maior ocorrência na faixa etária de 13 a 15 anos (Raslova, 2010).

Grande progresso tem sido feito sobre o prejuízo do diabetes em vários órgãos graças à utilização de modelos animais espontaneamente diabéticos (Ribeiro *et al.,* 2006; Yatoh *et al.,* 2006; Yono *et al*., 2008), tais como ratos propensos ao diabetes (*Diabetes prone bio breeding* –

BBDP/Wor) ou camundongos diabéticos não-obesos (*non obese diabetic* - NOD). Muitos estudos sobre esse tema (Lazarow, 1952; Ribeiro *et al.,* 2008; Jorge *et al.,* 2009) também se baseiam na indução experimental dessa doença pela administração de drogas diabetogênicas como a estreptozotocina e a aloxana (Fig. 1), que destroem as células β pancreáticas produtoras de insulina, mimetizando as condições do DM1 humano.

A estreptozotocina é seletivamente acumulada nas células β pancreáticas pelo transportador de glicose GLUT2 presente na membrana plasmática. O efeito diabetogênico é originado pela metilação do DNA que leva à morte das células β pancreática através de necrose (Lenzen *et al.,* 2008). Já a aloxana possui uma estrutura muito semelhante à glicose e por isso é facilmente transportada pelo GLUT2 ao interior celular. Seu efeito diabetogênico se dá por geração de espécies reativas de oxigênio (ROS) (Lazarow, 1952; Szkudelski, 2001; Lenzen *et al.,* 2008). Os níveis hiperglicêmicos nestes modelos experimentais são alcançados cerca de 10 a 12 horas após um curto período hipoglicêmico (Lenzen, 2008).

Ratos adultos diabéticos, cuja indução é efetuada por estreptozotocina, são um bom modelo de estudo do sistema reprodutor, pois eles exibem deficiências na função reprodutiva que muito se assemelham aos padrões descritos nos humanos (Stege & Rabe, 1997).



Estreptozotocina

Aloxana

Figura 1: Fórmulas estruturais das drogas diabetogênicas estreptozotocina e aloxan Modificado de Lenzen *et al.*, 2008.

2. Hiperglicemia e formação de produtos de glicação não-enzimática (AGE)

As complicações do diabetes são em grande parte decorrentes da hiperglicemia Isso ocorre, pois o aumento de glicose e de outros açúcares no plasma leva à glicação não enzimática de proteínas, lipídios ou DNA e à posterior formação de produtos finais de glicação não-enzimática (AGE) (Baynes *et al*., 1989; Stiit *et al.*, 1997). Uma das principais reações não enzimáticas é ligação que ocorre entre o oxi-grupo do açúcar e o amino grupo das proteínas (Monnier *et al.*, 1992; Deyl e Miksik, 1997). Os produtos de Amadori são o resultado do primeiro passo irreversível na cascata de reações entre os açúcares e as proteínas. Já os AGE, como a Nε-(carboximetil) lisina (CML) e Nε-(carboxietil) lisina (CEL), são as estruturas químicas finais derivadas de rearranjos dos produtos de Amadori (Denis *et al.,* 2002). A CML é o AGE mais abundante encontrado *in vivo* (Ikeda, 1996). A proteólise de proteínas danificadas por glicação, tanto no meio intra e extracelular, disponibiliza os AGE para novas reações com proteínas (Ahmed *et al.*, 2005). A renovação de moléculas modificadas por AGE ocorre via receptores de AGE (RAGE) presentes na superfície celular, mediando a endocitose e subseqüente degradação, tendo importante função no catabolismo. Esses produtos da glicação não enzimática, conseqüentes da hiperglicemia, são acumulados desde o início do desenvolvimento embriológico e se formam durante processos metabólicos normais e sendo também introduzidos no corpo, via uma variedade de rotas exógenas incluindo dieta e fumo (Mallidis *et al.,* 2007).

Os AGE podem ocasionar mudanças físicas nos tecidos através de ligações cruzadas com proteínas teciduais como o colágeno, lipoproteínas plasmáticas e imunoglobulinas (Ha *et al.,* 2004). Muitos estudos têm apontado ainda que os íons de metais de transição são capazes de se ligar a proteínas glicadas, já que estas adquirem uma afinidade pronunciada por íons metálicos, como cobre e ferro (Saxena *et al.*, 1999). Outro prejuízo resultante da hiperglicemia e do aumento dos AGE nos tecidos relaciona-se à indução da formação de espécies reativas de oxigênio (ROS) e o conseqüente aumento do estresse oxidativo. A hiperglicemia favorece ao estresse oxidativo por meio de três mecanismos: (1) glicação não enzimática e formação dos AGE; (2) ativação da proteína quinase C (Koya & King, 1998) e (3) aumento da via dos polióis por ação da aldose redutase (Willimason *et al.*, 1991).Tem sido constatado que a formação de ROS pelos AGE depende ligação com seus receptores (RAGE) e subseqüente ativação de

NADPH oxidase (Yan *et al.,* 1994; Wautier *et al.,* 2001). Conforme ilustrado na Figura 2, a ativação de RAGE pode também alterar a transdução de sinais e processos genômicos, ocasionando a expressão de genes pró-inflamatórios (Du *et al.*, 1998; Waltier *et al.*, 2001) e a indução de apoptose (Naruse *et al.*, 2000; Denis *et al.*, 2002). A interação AGE-RAGE tem sido correlacionada à formação e progressão de tumores, visto que acentua também a síntese de fatores de crescimento e citocinas importantes para o crescimento celular, bem como de metaloproteinases de matriz (como MMP-2 e MMP-9) e da proteína quinase ativada por mitógeno (MAPK) envolvidas na invasão e metástase de células cancerosas. (Singh *et al.,* 2001; Kuniyasu *et al.,* 2002). Estudos mostraram que o bloqueio do sinal de RAGE inibe o crescimento e a metástase de células de glioma (Taguchi *et al.,* 2000).

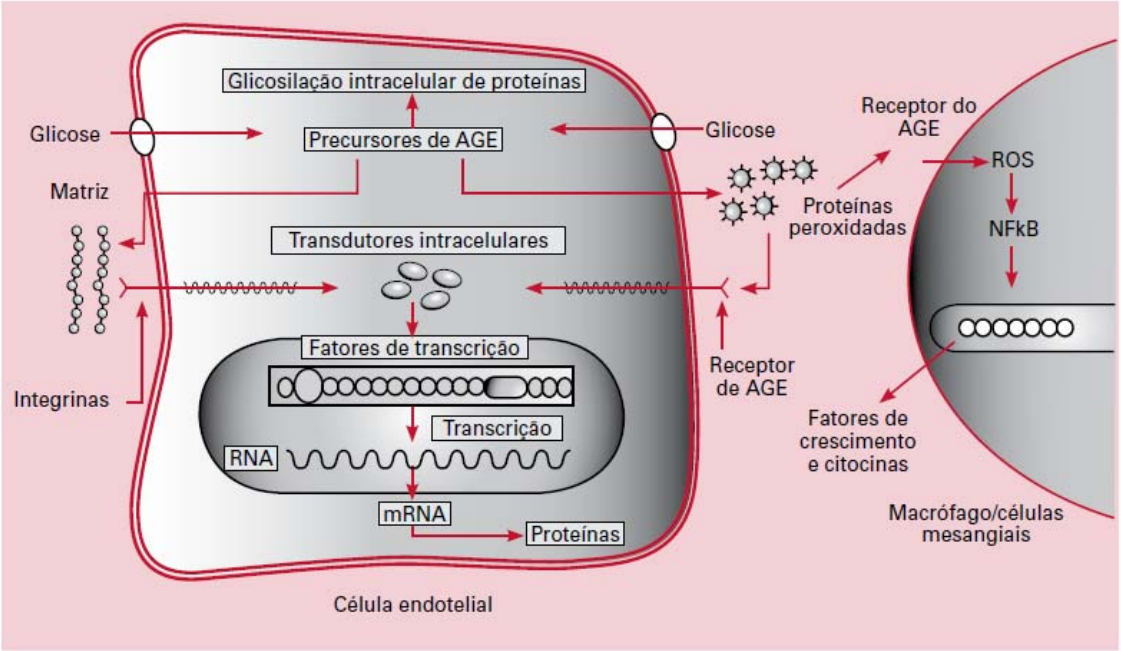


Figura 2: Representação esquemática Aumento da produção de AGE e suas conseqüências patológicas. Acredita-se que os AGE atuem modificando: proteínas intracelulares envolvidas na regulação gênica; moléculas da matriz extracelular vizinhas, interferindo na sinalização entre a matriz e a célula, causando disfunção celular; proteínas, como a albumina, que então ativam os receptores de AGE (RAGE), estimulando a produção de citocinas inflamatórias como a interleucina 1 e 6, fator de crescimento I, fator de necrose tumoral alfa, prostaglandinas e fator estimulador de colônias de granulócitos. NFκB - fator de transcrição kappa B.(Extraído de Reis *et al.*, 2008)

3. Espécies reativas de oxigênio (ROS) e estresse oxidativo

As espécies reativas de oxigênio (ROS) são átomos, íons ou moléculas que contêm oxigênio com um elétron não-pareado em sua órbita externa. São caracterizadas por grande instabilidade e elevada reatividade e tendem a ligar o elétron não-pareado com outros elétrons presentes em estruturas próximas de sua formação, comportando-se como receptores (oxidantes) ou doadores (receptores) de elétrons (Reis *et al.* 2008). Diversas vias podem dar origem às ROS, como a ruptura do transporte da cadeia de elétrons, lipoxigenases e as vias relacionadas à hiperglicemia (Willimason *et al.*, 1991; O´Donnell *et al.*, 1995; Yan *et al.,* 1994; Koya e King, 1998; Wautier *et al.,* 2001).Em baixas concentrações, as ROS estão envolvidas na regulação de vários processos fisiológicos tais como proliferação celular, diferenciação, apoptose e senescência, podendo atuar como mensageiros secundários nesses processos. Em altas concentrações, as ROS são extremamente tóxicas para a célula (Gupta *et al.,* 2004). A produção de ROS tais como peroxila (LO_2), radicais hidroxila (OH), peróxido de hidrogênio (H_2O_2), superóxido (O_2^-), oxigênio *singlet* entre outros, levam ao aumento da peroxidação lipídica, danos no DNA, degradação de proteínas e carboidratos, expressão gênica alterada e apoptose (Gupta *et al.,* 2003; Koyu *et al.,* 2006; Koyuturk *et al.*, 2006).

O estresse oxidativo consiste no desequilíbrio entre a formação e a remoção das ROS no organismo decorrente da menor formação ou maior consumo, ou do aumento da geração de espécies oxidantes (Oga, 2003).

Os antioxidantes são compostos que protegem sistemas biológicos contra efeitos potencialmente danosos de processos ou reações que promovem a oxidação de macromoléculas ou estruturas celulares. Dessa forma, o sistema de defesa antioxidante (AOS) é formado por uma ampla gama de substâncias tais como proteínas, enzimas, vitaminas e outros quelantes que atuam em diferentes níveis. O sistema de defesa primário constitui a primeira linha de defesa, formado por substâncias que impedem a geração de ROS, ou seqüestram-nas, de forma a impedir sua interação com alvos celulares. Nesta categoria encontram-se as enzimas antioxidantes, quelantes e proteínas como a transferrina e ceruplasmina, que transportam ferro e cobre, respectivamente, impedindo que esses metais sejam liberados e catalisem a formação de ROS, bem como substâncias não enzimáticas como o urato, ácido ascórbico (vitamina C), albumina, bilirrubina e

carotenóides. Essas últimas seqüestram radicais superóxido e hidroxila, ou suprimem oxigênio *singlet*. O sistema de defesa secundário é formado geralmente por compostos fenólicos ou aminas aromáticas, que atuam bloqueando a etapa de propagação da cadeia radicalar, seqüestrando radicais intermediários a exemplo da peroxila. Neste caso podemos mencionar os tocoferóis (vitamina E), tocotrienóis, flavonóides e vários antioxidantes sintéticos. Uma terceira linha de defesa antioxidante é caracterizada pelos sistemas de reparo do DNA, por proteases e fosfolipases, os quais atuam removendo as lesões oxidativas do DNA, proteínas e lipídios, respectivamente (Oga, 2003).

4. Enzimas antioxidantes

As enzimas antioxidantes combatem a toxicidade associada com os radicais livres e ROS (Sreepriya e Bali, 2006). Através da atuação das enzimas superóxido dismutase (SOD), glutationa peroxidase (GPx), catalase (CAT) e glutationas s-transferases (GST), as concentrações das ROS são mantidas muito baixas.

Existem três classes de SOD, a Fe-SOD que está presente em procariotos, a Mn-SOD e a CuZn-SOD que estão presentes nos eucariotos. As SOD dependentes de cobre e zinco encontram-se no citoplasma celular e fluido extracelulares. Já a Mn-SOD é uma enzima mitocondrial. A SOD age sobre o radical superóxido (O_2^-) e tem como produtos o oxigênio (O_2) e o peróxido de hidrogênio (H_2O_2) (Van der Oost *et al.,* 2003).

A redução de hidroxiperóxidos orgânicos e inorgânicos (H_2O_2) se dá pela GPx e é catalisada pela glutationa reduzida (GSH) para formar glutationa oxidada (GSSG) e água (ou alcoóis). A continuidade do ciclo catalítico da GPx depende da redução da glutationa redutase (GR), que utiliza NADPH formado pela via das pentoses. Esta enzima é encontrada no citoplasma e mitocôndrias (Zhang *et al.,* 2004).

A catalase (CAT) é a enzima presente em peroxissomos e microperoxissomos, sendo particularmente abundante em hepatócitos e células do rim. É uma hemeproteína contendo quatro grupos heme. Possui especificidade para o peróxido de hidrogênio, não atuando sobre peróxidos orgânicos produzidos pelo metabolismo de ácidos graxos (Zhang *et al.,* 2004).

As GST são um grupo de enzimas da fase II que catalisam a reação de conjugação da glutationa reduzida (GSH) com uma variedade de compostos eletrofílicos, envolvendo a detoxificação de intermediários reativos e ROS (Van der Oost *et al.,* 2003).

Estudos bioquímicos têm procurado avaliar as alterações nessas enzimas frente a situações de exposição a metais, outros tipos de contaminação e em algumas doenças (Gupta *et al.,* 2004; Zhang *et al.,* 2004; Klimzcak, *et al.,* 1984; Almeida *et al.,* 2004; Kotrikadze *et al.,* 2008). O maior número de estudos a esse respeito em sido direcionado para órgãos brânquias, rim, fígado (Klimzcak, *et al.,* 1984; Manca *et al.,* 1991; Bagchi *et al.,* 1997; Van der Oost *et al.,* 2003;) Pouco se sabe sobre o comportamento do AOS em órgãos do aparelho genital nas condições acima mencionadas.

5. Vitamina C

O ácido ascórbico ou vitamina C é uma lactona insaturada com uma estrutura de anel de cinco elementos (Fig. 3). Cada carbono está ligado a um grupo hidroxila, exceto pelo carbono carboxílico que está envolvido na ligação éster que torna a molécula cíclica. A maioria dos animais pode sintetizar a vitamina C, com exceção dos primatas e porquinhos-da-índia (Campbell, 2000.). Trata-se de uma vitamina hidrossolúvel que é encontrada em uma variedade de alimentos, mas particularmente em frutas e vegetais.

Essa vitamina age como um potente antioxidante nos fluidos biológicos por eliminar ROS fisiologicamente relevantes. Seu papel efetivo como antioxidante se dá pela interconversão através de um sistema de oxidação entre as duas principais formas; o L-ácido ascórbico e o dihidro-L-ácido ascórbico. Assim, o ácido ascórbico e seu radical possuem baixos potenciais redutores e podem reagir com a maioria dos outros radicais e oxidantes (Yasser *et al.,* 2002).

Outra função biológica da vitamina C é a sua interação com metais de transição que possuem atividade redox, como o ferro e o cobre. A vitamina C também age como um co-substrato para as enzimas prolil hidrolase e oxigenase, carnitina e neurotransmissores. A prolil hidrolase atua na conversão dos resíduos de prolina do colágeno em hidroxiprolina, portanto, a deficiência em vitamina C leva à fragilidade do colágeno, responsável pelos sintomas do escorbuto (Campbell, 2000.).

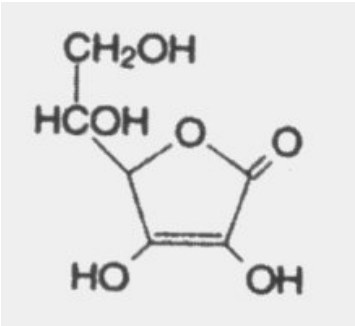


Figura 3. Fórmula estrutural da vitamina C (ácido ascórbico). Retirado de Campbel, 2000.

6. Morfologia e fisiologia prostática

A próstata é uma glândula acessória do sistema genital masculino que juntamente com a vesícula seminal contribui com a produção de nutrientes para o fluido seminal e promove manutenção do gradiente iônico e pH adequado para sobrevivência dos espermatozóides (McNeal, 1997; Untergasser *et al.,* 2005). Constitui-se de unidades glandulares túbulo-alveolares ramificadas com epitélio secretor pseudo-estratificado colunar (McNeal, 1997; Schaklen e Van Leenders, 2003). Em humanos, ela exibe uma morfologia compacta sendo diferenciada em três zonas: central, de transição e periférica (McNeal, 1997). Na maioria dos roedores, a glândula forma um complexo composto por quatro lobos distintos: anterior ou glândula coaguladora, dorsal, lateral e ventral (Hayashi *et al.,* 1991). Os lobos dispõem-se ao redor da uretra pélvica, na base da bexiga, exibindo particularidades quanto à ramificação de ductos e produção de secreções protéicas (Sugimura *et al.*, 1986; Rochel *et al.,* 2007). Ductos prostáticos de um mesmo lobo mostram heterogeneidade regional quanto à distribuição celular, síntese e secreção protéica bem como resposta a andrógenos (Banerjee *et al.*, 1998; Góes *et al.*, 2006).

Entremeando as porções glandulares há um estroma ricamente vascularizado, com moléculas reguladoras, enzimas de remodelamento, células do sistema imune, nervos, fatores de crescimento, além de fibras colágenas, elásticas, fibroblastos e células musculares lisas (SMC) (McNeal, 1997; Carvalho e Line, 1996). O colágeno é secretado pelos fibroblastos e SMC e tem papel estrutural, promovendo adesão celular, resistência e integridade do tecido. Já as fibras elásticas estão relacionadas à extensibilidade e à deformação prostática (Montes, 1992; Carvalho *et al.,* 1997b). Juntos, esses elementos microfibrilares têm importante função, fornecendo a

plasticidade celular necessária durante a contração da SMC e restauração elástica no retorno da contração (Carvalho *et al.,* 1997a). As SMC dispõem-se ao redor dos ductos e envolvem as unidades ducto-acinares, formando vários estratos celulares. Por tal disposição e sua capacidade contrátil, as SMC representam um papel crucial para a fisiologia prostática, pois promovem a rápida expulsão do sêmen.

Em todas as espécies, a próstata desenvolve-se a partir do seio urogenital endodermal (UGS). O desenvolvimento da próstata, assim como o de outros órgãos formados pela associação de um parênquima epitelial com um estroma fibromuscular, depende de interações recíprocas entre o epitélio em diferenciação e o mesênquima subadjacente (Marker *et al.*, 2003). A ação androgênica é fundamental ao desenvolvimento e manutenção da homeostasia prostática. Aproximadamente 90% da testosterona presente no plasma está ligada às proteínas séricas de ligação dos hormônios sexuais (SHBG) e apenas a testosterona livre pode entrar nas células prostáticas e ser convertida em dihidrotestosterona (DHT) pela enzima 5-α-redutase (Jena & Ramarão, 2010). O efeito androgênico é mediado pelos receptores de andrógenos (AR), cuja ativação leva à expressão de genes envolvidos com a atividade secretória, como o PSA (antígeno específico prostático), ou genes regulatórios de fatores de crescimento (Doncajour & Cunha, 1993). Sabe-se que, durante o desenvolvimento, as células mesenquimais são as primeiras a expressarem tais receptores (Cooke *et al.*, 1991) e então, sob o estímulo dos andrógenos liberam fatores parácrinos que agem sobre as células epiteliais induzindo a morfogênese ductal e a aquisição da sua identidade específica (Cunha e Lung, 1978; Sugimura *et al.,* 1986; Cunha *et al.,*1987; 1992; 2004). Vários estudos também indicam que os sinais parácrinos de origem epitelial, aliados aos andrógenos, influenciam o padrão de diferenciação das células estromais (Marker *et al.,* 2003). Assim, existem evidências consensuais de que a manutenção do fenótipo diferenciado das células epiteliais e estromais da próstata adulta é dependente de interações recíprocas entre essas populações celulares.

O desequílibrio das interações epitélio-estroma pode desempenhar um papel decisivo na instalação e progressão das doenças que acometem esse órgão como a hiperplasia benigna e o carcinoma de próstata (Cunha *et al.*, 1996; Hayward *et al.*, 1996). Portanto, considera-se de fundamental importância a execução de estudos específicos que permitam investigar os

mecanismos moleculares envolvidos nas interações epitélio-estroma na próstata normal e as possíveis alterações que ocorrem nos estados patológicos.

A formação do epitélio prostático, em roedores envolve a emergência de um epitélio sólido a partir do epitélio estratificado do seio urogenital e subseqüente canalização para formar ductos com epitélio colunar simples. A diferenciação ocorre durante a canalização e as proteínas secretoras próstata específicas são inicialmente detectadas de 12-20 após o nascimento (Hayward *et al*., 1996).

O epitélio secretor da próstata (Fig. 4) é composto por vários tipos celulares inter-relacionados como as células-tronco, as células basais, as células de amplificação transitória (*transit-amplifying cells* - TAC), as células intermediárias e as células luminais secretoras (Isaacs e Coffey, 1989; De Marzo *et al.,* 1998; Schaklen e Van Leenders, 2003). Esses representam estágios progressivos de diferenciação, sendo distinguidos não apenas quanto à morfologia e localização, mas também quanto ao padrão de expressão gênica, respostas a estímulos fisiológicos e plasticidade celular. As células luminais secretoras representam a maioria das células epiteliais da próstata normal e hiperplásica (Liu *et al*., 1997) e por se tratarem das células funcionalmente ativas do parênquima prostático, exibem abundância de organelas envolvidas com a síntese de secreção protéica (Garcia-Flórez e Carvalho, 2005). São células terminalmente diferenciadas e andrógeno-dependentes, sendo, portanto as mais afetadas em condições de carência androgênica que levam à apoptose (Aumuller, 1995). Outra linhagem de células desse epitélio acinar corresponde às células neuroendócrinas, cuja origem ainda é debatida, mas não estão sujeitas à ação androgênica (Schaklen e Van Leenders, 2003). A população de células basais é também pouco conhecida, mas sabe-se que parte delas representa um pool de células-tronco essenciais para a renovação epitelial (Garcia-Florez e Carvalho, 2005).

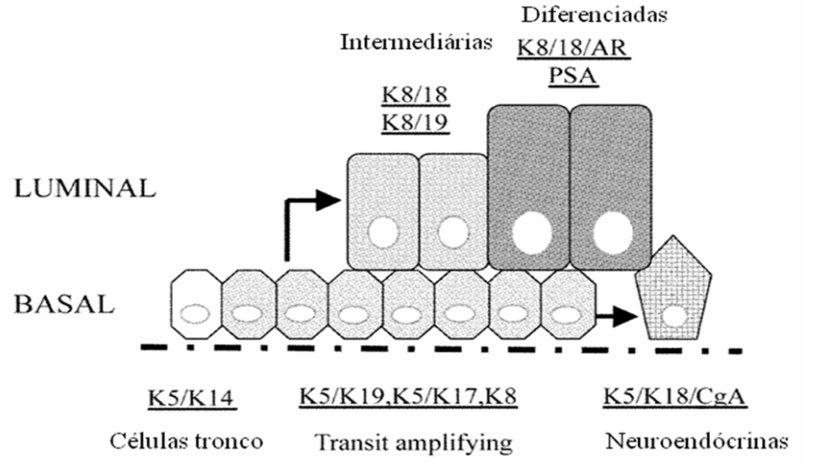


Figura 4: Possíveis rotas de diferenciação para células epiteliais da próstata humana baseados nos padrões de expressão de queratinas (K). Células-tronco basais (K5 e K14) aumentam uma população de células intermediárias expressando K19, K15 e K17. Essas células diferenciam-se em células luminais com expressão de K19 juntamente com K8/K18 antes de perderem a expressão de K19 e depois iniciam a secreção de PSA e se tornam terminalmente diferenciadas. Em uma via alternativa, células intermediárias começam a expressar cromogranina A (CgA) e diferenciam-se em células neuroendócrinas. (Modificado de Hudson *et al*., 2001 com permissão da Histochemical Society, 2002).

7. Problemática do estudo

Alguns efeitos nocivos do diabetes sobre as funções reprodutivas masculinas são bem conhecidos, como a impotência, a redução da libido e a esterilidade, essa última associada tanto à diminuição da quantidade e como da qualidade dos espermatozóides (Kolodny *et al*., 1874; Stege e Rabe, 1997; Scarano *et al*., 2006). Os efeitos prejudiciais sobre as glândulas acessórias sexuais também têm sido retratados. Estudos experimentais com roedores têm revelado que o diabetes leva a involução da próstata, atrofia epitelial e remodelação estromal com aumento de colágeno e proteoglicanos de condroitin sulfato, além de alterações fenotípicas e funcionais nas células estromais (Crowe *et al*., 1987; Cagnon *et al.,* 2000; Carvalho *et al.* 2003; Ribeiro *et al.,* 2006; 2009; Suthagar *et al.*, 2008; Gobbo *et al.,*submetido). Esses processos são acompanhados de desequilíbrio na cinética de proliferação e morte celular na glândula (Fávaro *et al.*, 2008; Arcolino *et al.,* 2010). A resposta prostática ao diabetes se explica, em grande parte, pela redução dos níveis de testosterona que ocorre nessa doença (Chandrashekar *et al.,* 1991; Yono *et al.* 2005), devido à interferência no eixo hipotálamo-hipófise-gonadal por redução na secreção do hormônio de liberação do hormônio luteinizante (LHRH).

Outros estudos têm procurado avaliar o papel da insulina nas alterações prostáticas frente ao diabetes. A insulina é um hormônio anabólico intrinsecamente relacionado à regulação do metabolismo e crescimento corporal (Saucier *et al.*, 1981; Anderson *et al.,* 1994) e que também influência a proliferação celular (Flakkol *et al.,* 2000). Sua ação é mediada por receptores específicos (IR) da membrana plasmática (Kahn, 1985) e envolve diferentes substratos intracelulares como IRS-1 and IRS-2 (Cheatham e Kahn, 1995) e vias de sinalização da PI3K (fosfatidil inositol 3-quinase) (Yenush e White, 1997) e da AKT (quinase de serina-treonina) (Downward, 1998; Chen *et al.*, 2001). A AKT fosforila diferentes substratos, como a Rheb e o

inibidor de rifampicina mTOR *(mammalian target of rapamycin)*, os quais estão envolvidos na síntese protéica (Inoki *et al*., 2003). A via mTOR é um importante regulador do crescimento e proliferação celular e desequilíbrios na sua regulação estão associados a doenças humanas como o câncer e o diabetes (Ueno *et al.*, 2005; Sabatini, 2006). Assim, essas vias regulam a intensidade e duração da ação da insulina e seu desequilíbrio acarreta intolerância à glicose, hiperinsulinemia e resistência a insulina (White, 2003).

O fato de a próstata expressar receptores para insulina (IR) salienta ainda mais a importância dos estudos dos efeitos do diabetes sobre esta glândula (Saucier *et al.,* 1981). Tem sido observado que a reposição de insulina potencializa os efeitos da administração de testosterona *in vitro* e normaliza tanto o peso prostático quanto os níveis séricos de testosterona em animais diabéticos (Calame e Lostroh, 1964; Lostroh, 1970; Wang *et al.,* 2000; Sudha *et al.*, 2000; Suthagar *et al.,* 2008). A insulina também estimula o crescimento e proliferação de linhagens de células prostáticas de rato *in vitro* (Polychronakos *et al.*, 1991; Antonioli, 2003). Análises prévias demonstraram que o tratamento de ratos diabéticos por aloxana com insulina mantém os níveis de testosterona circulante, bem como preserva parcialmente a morfologia de células estromais, em especial dos fibroblastos (Arcolino *et al.,* 2010; Gobbo *et al.,* submetido). Também foi constatado nesses estudos que a reposição de insulina protege contra os desequilíbrios nas taxas de apoptose, mas não afeta os níveis de proliferação celular (Arcolino *et al.,* 2010). O tratamento concomitante de ratos NOD com insulina, testosterona e estrógenos restaura as modificações estruturais da próstata, mas não normaliza totalmente a cinética de proliferação e morte das células epiteliais (Fávaro *et al.,* 2008). Assim outros fatores além da falta de insulina e o desequilíbrio hormonal estão envolvidos na atrofia e nas alterações da proliferação e morte celular intra-epiteliais prostáticos, constatados no diabetes e provavelmente são oriundos da hiperglicemia

Em estudo anterior desenvolvido por Ribeiro e cols. (2008), constatou-se que uma ampla variação no grau de atrofia e nas alterações histológicas observados na próstata ventral de ratos, após três meses de diabetes induzido por aloxana e sem tratamento com insulina. Segundo esse estudo, 64% dos animais apresentaram drástica atrofia da próstata e 35% exibiram lesões neoplásicas (Ribeiro *et al.*, 2008). Além disso, 7% dos animais desse estudo apresentaram atrofia

prostática proliferativa (PIA), um padrão de alteração histológica que favorece a malignidade (De Marzo *et al.*, 1999). Dados de outros autores resultantes da comparação do diabetes euglicêmico e hiperglicêmico indicaram que essa última condição altera a expressão de alguns fatores envolvidos na regulação da proliferação e morte celular, estando diretamente implicada com as alterações prostáticas nessa doença (Yono *et al.,* 2008). Conforme mencionado anteriormente, os processos de proliferação e morte celular na próstata são direta ou indiretamente regulados por andrógenos. Considerando que a maior parte das neoplasias prostáticas são de natureza epitelial (Cunha *et al.,* 2004), torna-se fundamental conhecer os mecanismos que controlam esses processos em situações normais ou de distúrbios metabólicos, como o diabetes.

Estudos em outros sistemas biológicos têm tornado evidente que as complicações histopatológicas decorrentes do diabetes envolvem múltiplos fatores, muitos deles relacionados à hiperglicemia prolongada (Naruse *et al.*, 2000; Denis *et al.*, 2002; Ribeiro *et al.,* 2006; Lamers *et al.,* 2007). Investigações sobre a retinopatia diabética têm revelado que a hiperglicemia em si e vários produtos de glicação formados secundariamente estão implicados na apoptose dos pericitos da retina (Naruse *et al.*, 2000; Denis *et al.*, 2002). Um aumento de subprodutos do estresse oxidativo como ceramidas e diacilglicerol (Denis *et al.*, 2002), bem como a diminuição da atividade e dos níveis das enzimas antioxidantes têm sido relatado em quadros hiperglicêmicos (Chen *et al.,* 2006; Jorge *et al.,* 2009). Tem sido demonstrado que vários tipos de antioxidantes, como as vitaminas C e E, previnem as alterações biológicas relacionadas à hiperglicemia, tais como a expressão de citocinas e a renovação celular (Koya *et al*., 1997; Studer *et al*., 1997; Cameron e Lotter, 1999; Abiko *et al*., 2003; Rahimi *et al.*, 2005; Fernandes *et al*., 2009). Esses estudos evidenciam que os antioxidantes podem prevenir e até mesmo reverter os danos iniciais do diabetes. Além disso, tem sido cada vez mais constatado a influência dos ROS na carcinogênese em diversos tecidos (Spector, 2000; Gago-Domingues *et al.,* 2005; Kotrikadze *et al.,* 2008).

Análises do efeito do diabetes com estreptozotocina sobre os biomarcadores de estress oxidativo em órgãos do sistema reprodutor masculino como o testículo e o epidídimo, mostram um aumento em comum da peroxidação lipídica e da atividade de enzimas antioxidantes como as GST (Shrilatha e Muralidhara, 2007a; 2007b; Armagan *et al.,* 2006). Foi demonstrado ainda por

esses mesmos estudos que o tratamento com antioxidantes como a melatonina e a vitamina C atenuam os danos do estress oxidativo, no diabetes induzido por estreptozotocina, diminuindo os níveis de produtos da peroxidação lipídica e restaurando a atividade enzimática de antioxidantes. (Shrilatha e Muralidhara, 2007a; Armagan *et al.,* 2006). Porém, existem poucos dados bioquímicos a respeito das variações nos marcadores de estresse oxidativo na próstata frente ao diabetes, seja em estudos experimentais ou clínicos (Kotrikadze *et al.,* 2007; Sarafinovska *et al.,* 2009). A maior parte das investigações que procuram esclarecer a relação de causa-efeito entre o câncer de próstata e o dano oxidativo foram baseadas em análises dos biomarcadores de estresse no sangue e não na própria glândula (Kotrikadze *et al.,* 2008; Sarafinovska *et al.,* 2009). Análises imunohistoquímicas mostram que as enzimas antioxidativas e produtos de estress são mais expressos no câncer de próstata metastático (Oberley *et al.*, 2000). Evidências de estudos experimentais com roedores têm indicado um aumento da incidência de neoplasias malignas e pré-malignas na próstata de animais diabéticos (Cagnon *et al.,* 2000; González-Pérez e Garcia Rodriguez, 2005, Burke *et al.*, 2006; Ribeiro *et al.,* 2008; 2009). Foi constatado que a alteração no balanço entre andrógenos e estrógenos pode favorecer o aparecimento de doenças na próstata (Risbridger *et al.,* 2003). Entretanto, apesar do progresso no entendimento das conseqüências dessa doença sobre a próstata, as interferências da hiperglicemia e seus produtos como AGE e ROS na diferenciação epitelial e as possíveis relações com a incidência de lesões malignas na glândula ainda não são claras.

Visto que a testosterona é um fator trófico essencial para a próstata, a apoptose foi classicamente descrito neste órgão com o uso de experimentos de castração (Kerr e Seale, 1973, Kyprianou *et al.,* 1988). Portanto, a involução prostática causada pela privação androgênica é devida em grande parte à perda das células luminais secretoras por apoptose (Kerr e Seale, 1973, Isaacs, 1989; Kyprianou *et al.,* 1988, Banerjee *et al.*, 2000; Staack *et al.,* 2003). O pico de apoptose ocorre 3-4 dias após a castração (Kyprianou *et al.,* 1988; García-Flórez e Carvalho, 2005). De acordo com Kyprianou *et al.* (1988), após dez dias de supressão androgênica causada pela castração, a próstata ventral involui completamente com perda de 80% das células epiteliais andrógeno-dependentes. Com o tempo, os níveis de apoptose retornam ao normal, uma vez que as células sobreviventes são andrógeno-independentes. A indução da apoptose causada pela

supressão de testosterona pode ser explicada indiretamente pelo comprometimento da produção de fatores de crescimento produzidos pelo estroma ou diretamente devida à produção de sinais estimulatórios da apoptose (Kurita *et al.,* 2001).

Conforme já mencionado sabe-se que o diabetes interfere na cinética da proliferação e morte celular no epitélio acinar da próstata (Fávaro *et al.,* 2008; Arcolino *et al.,* 2010). É bem estabelecido que o diabetes leva a uma diminuição acentuada dos níveis séricos de testosterona (Yono *et al.,* 2005) e na expressão de AR Os níveis de apoptose estão aumentados nessa situação (Fávaro *et al.,* 2008), permanecendo altos em situações de diabetes crônico não tratado (Arcolino *et al.,* 2010). Ao contrário do observado para animais castrados (Oliveira *et al.,* 2007; Marks *et al.,* 2008), a administração de testosterona não é capaz de normalizar os níveis de apoptose (Favaro *et al.,* 2008). Além disso, nem mesmo a administração conjunta de testosterona, insulina e estrógeno é capaz de normalizar esses níveis (Favaro *et al.,* 2008). Não existe consenso sobre os efeitos do diabetes na proliferação do epitélio prostático visto que Fávaro *et al.* (2008) constaram um aumento de células ki-67 positivas e outros resultados mostram o oposto quando avaliado a freqüência de células marcadas pela PCNA (Arcolino *et al.,* 2010; Costa *et al.,* submetido). Em experimentos anteriores com o diabetes induzido experimentalmente em ratos pela administração de aloxana e mantido sem reposição de insulina durante três meses temos observado uma variação individual na resposta da próstata frente a esse distúrbio (Ribeiro *et al.*, 2008, 2009). Nesses estudos constatamos que para a maior parte dos animais o diabetes induz severa atrofia prostática, em uma pequena parte a próstata se mantém praticamente inalterada e em um terço deles ocorre lesões malignas

Os dados acima mencionados, permitem supor que outros fatores estão implicados no aumento da apoptose e alterações teciduais dessa glândula no diabetes, um dos quais pode ser a hiperglicemia e os produtos dela resultantes como os AGE. Além disso, considerando que os fatores envolvidos nas variações inter-individuais observadas na resposta da próstata ao diabetes ainda não são totalmente compreendidos, nos propusemos a avaliar se existe correlação entre a concentração sérica de testosterona, o grau de hiperglicemia e o grau de involução da próstata de ratos em modelo de diabetes induzidos por aloxana.

Objetivos

O presente estudo teve como objetivos:

- Investigar o impacto do diabetes induzido por estreptozotocina sobre o sistema antioxidante da próstata e do sangue, além do possível papel protetor da vitamina C frente aos produtos da hiperglicemia (AGE) e o estresse oxidativo. Avaliar a possível influência do estresse oxidativo sobre as taxas de proliferação e morte celular das células do epitélio prostático e a sensibilidade androgênica dessa glândula
- Esclarecer os mecanismos envolvidos nas variações individuais na resposta da próstata frente ao diabetes induzido por aloxana em curto prazo e o papel da insulina em prevenir as alterações morfológicas iniciais. Correlacionar essas alterações histológicas com os níveis séricos de testosterona e estrógeno em diferentes faixas glicêmicas.

Resultados

Os dados do presente trabalho foram reunidos em dois manuscritos:

Manuscrito 1: Biomarkers of oxidative stress in the prostate of diabetic rats.

Manuscrito 2: Inverse relation between glycemia and testosterone/estradiol ratio modulates histological changes in the ventral prostate of diabetic rats.

Biomarkers of oxidative stress in the prostate of diabetic rats

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Running Title: Oxidative stress in prostate of diabetic rats

Key words: experimental diabetes, oxidative stress, prostate, vitamin C supplementation, apoptosis

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Abstract

Background: Negative repercussions of diabetes on the prostate are associated with diminished testosterone, insulin lack and hyperglycemia. The contributions of oxidative damage, which usually increases with diabetes, are unknown for these alterations. This study evaluated the impact of streptozotocin-induced diabetes (STZ-D) on biomarkers of the antioxidant system of rat ventral prostate and the influence of vitamin C supplementation as well as the possible correlations with unbalanced cell proliferation and death.

Methods: Male Wistar rats were injected with streptozotocin (5mg/100g b.w., i.p.) for diabetes induction and treated with vitamin C (150mg/kg b.w./day) via gavage (D+V) or water (D) for 30 days. Control groups received vitamin C (C+V) or the vehicle only (C). Prostate was analyzed using light microscopy, immunocytochemistry and specific biochemical essays for biomarkers of oxidative stress.

Results: Both D and D+V (hyperglycemia around 500 mg/dL) showed low circulating testosterone and reduced number of androgen-responsive cells. Cell proliferation decreased and apoptosis increased in diabetic groups regardless of vitamin C treatment. Superoxide dismutase and glutathione peroxidase did not change but catalase and gluthatione S-transferase (GST) increased in the prostate after STZ-D. Malondialdehyde and the AGE Nε-(carboxymethyl) lysine also increased marginally in STZ-D. Vitamin C supplementation normalized GST activity and recovered the apoptotic rates in the prostate**.**

Conclusions: These results demonstrated that oxidative damage is, at least in part, responsible for unbalanced cell proliferation and apoptosis observed in the rat prostate after diabetes and GST is a good indicator of compensatory oxidant defense in the gland at earlier stages of such metabolic disease.

Introduction

Diabetes is a complex disease with multiple etiologies characterized by hyperglycemia and insufficiency of insulin secretion or action. Male reproductive function is impaired in diabetes and there are increasing evidences to indicate that prostate gland is also negatively affected. Experimental studies demonstrated that diabetes led to prostatic involution [1 - 3], imbalance in kinetics of proliferation and cell death in the secretory epithelium [4 - 6], extracellular matrix remodeling as well as functional and phenotypic changes in stromal cells [3, 6].

Most of the above mentioned studies were based on spontaneously developed or drug-induced diabetes and emphasize the short-term alterations in prostate. Despite some differences among animal models of diabetes, the histopathological changes caused in the prostate by this metabolic disturbance have been usually related to testosterone drift; however, others factors such as impaired insulin action, TGF-ß up-regulation and IGF have also been implicated [2, 3, 5, 6]. Data from Fávaro *et al.* [5] with non-obese diabetic mice (NOD) and ours with alloxan-induced diabetes [6] have shown that regardless of the diabetes model, diabetes increase the apoptotic rates in prostate. Meanwhile, there is no consensus concerning its consequences on cell proliferation and an inhibitory [6] or stimulatory effect has been reported [5]. Although these discrepancies may be explained by differences in experimental models, period of the disease and analytical methods, not even the associated administration of testosterone and insulin was able to normalize the cell proliferation levels in NOD mice [5]. Thus more information is necessary to understand the impact of diabetes in proliferative activity of prostate.

Evaluation of the prostate response after three months of alloxan-induced diabetes without insulin replacement, revealed a raise in the incidence of prostatic intraepithelial neoplasia (PIN) and malignant neoplasia [7, 8]. However the association between diabetes and prostate cancer in clinical studies is still a matter of debate [9, 10]. Thus, the analysis of prostate alterations during diabetes progression becomes an important issue.

Several complications of diabetes are due to hyperglicemia. Comparisons of molecular changes in prostate of insulin-treated diabetic rats by means of microarray analysis have indicated that hyperglycemic diabetes altered the expression of some factors involved in cell proliferation

and apoptosis which are not affected in the euglycemic condition [11]. The rise of glucose and other sugars in plasma leads to non-enzymatic glycation of proteins, lipids and DNA, which form the end products of non-enzymatic glycation (AGE) [12]. One of the most common non-enzymatic reactions is the link between the oxy group of the sugar and the amino group of proteins [13], forming the Amadori products, which undergo additional rearrangements and originate the AGE Nε-(carboxymethyl) lysine (CML).

The hyperglycemia and AGE increases are directly associated with the formation of reactive oxygen species (ROS) and prooxidant state. Studies in other organs have provided compelling evidence that the prooxidant state may lead to apoptosis [14 - 16]. A prooxidant state has also been associated with carcinogenesis due to DNA damage, interaction with oncogenes or tumor suppressor genes and immunological mechanisms [17, 18]. In the specific case of prostate, the protective role of some antioxidants such as carotenoids, vitamin E and selenium against malign transformation has been proven [19 - 21]. In addition, alterations in the biomarkers of oxidative stress have been demonstrated in the blood of patient with prostate cancer [18, 22]. However, information about the behavior of the antioxidant system in this tissue under pathologic conditions is scarce [23, 24]. Besides, little is known about the contribution of oxidative stress in the prostate response under diabetes.

Considering the pivotal role of oxidative stress in complications of diabetes and malignancies, the present study examined the repercussions of streptozotocin-induced diabetes on antioxidant system (AOS) of rat ventral prostate and the influence of concomitant vitamin C supplementation in this situation. The possible correlation between oxidative stress and the changes in cell proliferation and death and androgenic sensitivity in experimental diabetes were also assessed.

Material and methods

1. Experimental design

Experiments with animals were approved by the institutional Ethical Committee for Animal Experimentation (UNESP, Protocol no. 006/09-CEEA) and they were in accordance to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of

Health. Adult male Wistar rats (3 months old) were provided by the breeding house of São Paulo State University (Botucatu, SP, Brazil) and they were kept at 24 °C on a 12h light / dark cycle and had free access to food and water for one week before the beginning of the experiments. Rats were randomly divided into four groups (n = 15 per group): control (C), treated with vitamin C (C+V), diabetic (D) and diabetic treated with vitamin C (D+V).

The induction of diabetes was made after 24 hours of fasting using intra-peritoneal injection of 5mg/100g body weight of streptozotocin (Sigma, St. Louis, MO, USA) diluted in citrate buffer 0.01 M, pH 4.5. Control animals were injected only with citrate buffer. Blood glycemia was evaluated in the finger tips using the glucose monitor Accu-chek (Roche Diagnostics, Mannheim, Germany). Only animals showing blood glucose levels above 200 mg/dL were included in the diabetic groups.

Vitamin C (ascorbic acid - Sigma Chemical Co., St Louis, MO, USA) was administered in control and diabetic animals by gavage (150mg/kg body weight/day) diluted in water, during 30 days. Food and water intake, as well as body weight were monitored along the experiments. Rats were killed one month after diabetes diagnosis and treatment, by CO_2 inhalation and decapitation.

2. Hormone dosages

The blood was collected in polyethylene tubes containing heparin immediately after decapitation of rats and centrifuged to separate plasma. Plasma testosterone and estradiol were measured using Modular Analyzer for Immunoassay of Chemiluminescence ECI (Johnson and Johnson, Langhorne, PA, USA), as described by Weeks and Woodhead [25]. Ten rats were used from each group, and the test was performed in triplicate. The intra- and inter-assay variations were 4.6% and 4.3% respectively.

3. Light Microscopy

After removal and weighing of the ventral prostate (VP), the right ventral lobe was isolated and some fragments were fixed by immersion in Karnovsky solution (2.5% freshly prepared formaldehyde, 2.5% glutaraldehyde in Sörensen phosphate buffer pH 7.2) for 12-24h, for processing in paraffin and historesin embedding (Historesin Embbedng Kit-Leica). Histological sections were stained with hematoxylin-eosin for general morphological

study. Other fragments were fixed by immersion in 4% formaldehyde freshly prepared in phosphate buffer pH 7.2 and in Methacarn (1:3:6 of acetic acid, chloroform and methanol) and they were used for immunocytochemical analysis. The analysis was made with a bright field microscope (Olympus CH30) coupled with a CCD camera and the digitization of selected microscopic fields as well as quantitative analyses were performed using an image analysis system (Image-Pro Plus Media Cybernetics,version 6.0 for Windows software, Betherda, MD, USA).

4. Immunocytochemistry

Immunocytochemical reactions for identification of CML [Nε-(carboxymethyl) lysine], a type of AGE (ab-30917, Abcam, USA), PCNA – the proliferating cell nuclear antigen - (sc-56, Santa Cruz Biotechinology, USA) and the androgen receptor, AR (sc-816, Santa Cruz Biotechinology, USA) were carried out. Sections were subjected to antigen retrieval in citrate buffer pH 6 for 20-40 minutes in the vegetabe steamer and immersed in 3% H_2O_2 in methanol to block endogenous peroxidase. Then, they were treated with 3% BSA in PBS in the case of CML and AR or Background Sniper (Biocare Medical, Concord, CA, USA), for PCNA reaction, to eliminate unspecific binding. After washing in PBS, the slides were incubated with primary antibodies diluted in 1% BSA as follows: mouse anti-human CML (1:125, overnight, at 4°C), mouse anti-human PCNA (1:50, 1h, at 37 º C) and rabbit anti-human AR (1:100, overnight, at 4°C). The detection of primary antibody was performed with peroxidase-conjugated polymer (Polymer Novolink, Novocastra, Norwell, MA, USA) following the incubation with diaminobenzidine (0.03% in TBS). Sections were stained with hematoxylin. Ventral prostate from rats 3 months after diabetes were used as positive control of CML. The negative control for all reactions was achieved by omitting the primary antibody.

5. Detection of apoptotic cells

Apoptotic cells were detected *in situ* using the DNA fragmentation essay associated to cell death based in TUNEL reaction (TdT-Fragel- Calbiochem, CN Biosciences, La Jolla, CA, USA), following the manufacturer instructions. Briefly, after digestion with proteinase K (1:100

in 10mM Tris pH 8.0) at room temperature for 23 min, slides were immersed in a solution of 3% H_2O_2 in methanol for 5 min, to block endogenous peroxidase. In a next step, they were incubated with biotinilated TdT followed by the enzyme deoxinucleotidil terminal transferase (TdT), for 1h 37°C. At the end of the reaction, the biotinilated nucleotides were detected by streptoavidin conjugated to peroxidase and the reaction was revealed using diaminobenzidine (0.07% in distilled water). Slides were finally stained with hematoxylin. Negative controls were obtained omitting the incubation with TdT enzime.

6. Quantification of CML positive areas, PCNA, TUNEL and AR positive cells

The images of immunoreactions and the TUNEL method were digitalized using the image analysis system described before. Five animals per group and three prostate fragments per animal were used. The CML positive areas were estimated using the point counting method of Weibel [26] with a 220-point reticulum and 20 contiguous microscopic fields per tissue section using the objective of 40X. The quantification of PCNA-, AR- and TUNEL-positive cells in the acinar epithelium of the intermediate region of the VP was determined as the number of positive nuclei divided by the total number of epithelial cell nuclei, using 20 contiguous microscopic fields and the 20X objective. The numeric data were expressed as relative frequency (%).

7. Activity of antioxidant enzymes

The left VP lobes were weighed and homogenized with 1:5 vol of buffer (Tris HCl 50, 1 mM EDTA, 1 mM DTT, 0.5 M sucrose, 0.15 M KCl, pH 7,4) and centrifuged at 10 000g for 20 min at 4ºC. The supernatant was centrifuged at 40000g for additional 60 min at 4ºC and the supernatant fraction was collected and used for the measurement of catalase (CAT), gluthatione S-transferase (GST), gluthatione peroxidase (GPx) and superoxide dismutase (SOD) activities.

Blood samples were collected immediately after sacrifice of the animals in polyethylene tubes containing heparin. For determination of CAT activity the blood samples were diluted at 50 times in distilled water, while this dilution was at 20 times in a hemolysing solution for GST and GPx activities.

Total protein content in samples was determined by the modified method of Lowry, using bovine serum albumin as standard [27].

CAT activity was quantified at 240 nm by the H_2O_2 decomposition according to the method of Beutler [28]. GST activity was determined by measuring the increase in absorbance at 340 nm, incubating reduced glutathione (GSH) and 1-chloro-2,4-dinithrobenzene (CDNB) as substrates, according to [29]. GPx activity was assayed by the oxidation of NADPH (linked to GSSG reduction by excess glutathione reductase) at 340 nm, as described by [30]. The SOD activity was measured at 550 nm by the method of [31] based on a system which generates superoxide (xanthine/xanthine oxidase) coupled to the reduction of cytochrome c by this radical. Thus, the SOD in the samples competes with cytochrome and by superoxide, inhibiting its reduction. The molar extinction constant (□) was used to calculate the activity levels of the antioxidant enzymes as U/mg protein (□ = 0,071 for CAT; □ = 6,22 for GST and □ = 9,6 for GPx).

8. Determination of lipid peroxidation levels

Malondialdehyde (MDA) level, an indicator of free radical generation, which increases at the end of lipid peroxidation, was estimated using the double heating method of Draper and Hadley [32]. For the MDA quantification in VP 100 µL of the resulting solution from tissue homogenization in buffer (1:5 vol) prepared as described in item 7 was used. Quantification of lipid peroxidation in blood was performed in the serum separated by centrifugation. Then 300 µL of 0.4% thiobarbituric acid solution diluted in 0.2 M HCl were added to the samples and incubated for 60 minutes at 90 ºC. After this period, the colored derivative of MDA-TBA was extracted with 1 mL of n-butanol followed by centrifugation and quantification at 532 nm. The data were expressed as nmol TBARs/g tissue and TBARs /mL plasma.

9. Statistical analysis

The data were presented as mean ± standard deviation. Statistical analyses were performed using ANOVA followed by tests for multiple comparisons using Statistica 6.0

Software (Copyright©StatSoft Inc., Tulsa, OK, USA). P values below 0.05 were considered statistically significant.

Results

Biometric and physiological data are shown in Table I. Diabetic rats showed a marked decrease in body weight, regardless of treatment with vitamin C. The same was observed for the prostate weight, thus the prostate relative weight did not vary between groups. Glucose levels were approximately four times higher in D and D+V in comparison with C, being above 480 mg/dL. Ratio intake did not differ between groups, but water intake was much higher for the diabetic groups.

Tissue alterations caused in ventral prostate by experimental diabetes with or without the vitamin C treatment are summarized in Figure 1. After one month of streptozotocin-induced diabetes the prostate atrophy was evident since the most acini exhibited reduced lumen, acinar epithelium with cubic cells and signs of epithelial shrinkage (Fig. 1E, F and G). The treatment with vitamin C did not affect the histology of prostate neither of control nor diabetic groups (Fig. 1A, B, H, I and J).

The immunocytochemical reaction for CML, the most abundant AGE *in vivo*, showed strong labeling in the ventral prostate of one month D compared to C and C+V groups (Fig. 2A, B, C, D and E). This AGE accumulated predominantly in acinar epithelium, particularly in nuclei of epithelial cells (Fig. 2C). The positive control for this immunoreaction was provided by the prostate from rats with chronic diabetes (three months of untreated diabetes) in which CML was more abundant and spread (Fig. 2E). In this case, CML deposition was also verified in apical surface of acinar epithelium and in vascular endothelium. The areas exhibiting CML accumulation diminished in the prostate of vitamin C treated diabetic rats (Fig. 2D and F). The relative frequency of CML immunostaining areas wasn´t statistically different among the experimental groups in this study.

Figure 3 shows the changes in biomarkers of oxidative stress in tissue samples and blood. No alterations were observed in blood, except for CAT activity in D+V (Fig. 3B, D, F and I). The CAT activity also increased in prostate tissue of diabetic rats independently of vitamin C administration (Fig. 3A). Treatment of diabetic rats with vitamin C restored the levels GST

activity in prostate which were elevated by diabetes (Fig. 3C). The prostatic levels of GPx and SOD activities (Fig. 3E and G) were not discrepant between groups, being uninfluenced by diabetes and treatment with vitamin C. The lipid peroxidation, evaluated by MDA levels, showed a discrete increase in the prostate of diabetic rats although it was not significant (Fig. 3H).

There was a reduction in number of AR-positive cells in D and D+V groups (Fig. 4C, D and E). As expected, diabetes led to a drastic reduction in circulating testosterone being about 10% of the C levels, in the case of D and 2.7% in D+V, showing less varied values in both diabetic groups (Table 1, Fig. 4F). The plasma estradiol did not vary greatly between groups and the untreated diabetic group showed a bigger individual variation of values (Table 1, Fig. 4G).

The TUNEL assay and quantitative analyses (Fig. 5A-D) showed that apoptotic cells were more abundant in diabetic rats where they were usually found in detached epithelial fragments in the acinar lumen (Fig. 5A, C and I). The treatment of diabetic rats with vitamin C restored the apoptosis to levels similar to control rats (Fig. 5A, D and I). The immunocytochemistry for PCNA indicated a marked reduction in cell proliferation levels in the acinar epithelium of the ventral prostate after one month of diabetes (Fig 5E, F, G and H). Furthermore, this reduction was not influenced by vitamin C supplementation (Fig. 5I and J).

Discussion

The oxidative stress-derived damages of diabetes are well described in several organs [14-16, 33-35] but to the best of our knowledge it has not been examined specifically in the prostate. In order to clarify this point we investigated the influence of streptozotocin-induced diabetes on biomarkers of oxidative stress, the CML adducts of proteins and kinetics of cell death and proliferation in this gland. The streptozotocin-induced diabetes is broadly accepted as an effective model of experimentally-induced diabetes type 1 and was chosen because unlike alloxan, diabetogenic action of streptozotocin is not due to the formation of ROS, but to the DNA alkylation of pancreatic β cells [36].

Most reports concerning the impact of diabetes in prostate are focused on short-term changes, and they showed that testosterone drift is the main responsible factor for gland atrophy

and disturbance of proliferation and cell death [1, 3, 5, 6, 37]. It also applies to this medium-term evaluation, since a drastic androgen reduction observed to D and D+V groups paralleled the reduction of androgen-responsive cells and cell proliferating levels. Diabetes stimulates apoptosis in several organs [14, 16] including the prostate [5, 6]. Earlier data from our laboratory revealed that apoptotic rates in the rat ventral prostate increase with disease progression [6] which raised the hypothesis that such alteration may be due to the progressive complications of this disease, such as hyperglycemia and in oxidant condition. The increase of apoptotic levels, observed in the present study, was coincident with biochemical data on MDA and immunocytochemical observations of CML which showed an increment of oxidative stress and AGE, respectively. It should be noted that in D and D+V groups the glycemia was high (~480mg/dL). Then, the present results suggest that, in addition to the androgen reduction, oxidative stress also contributes to imbalance of cell death and proliferation in the prostate. Higher apoptotic rates in comparison with those observed here were detected by Fávaro and cols. [5] in NOD mice after 20 days of diabetes which exhibited very high glicemia (~910mg/dl). Furthermore, not even the treatment with insulin, testosterone and estrogen in association were able to restore glycemia or apoptotic rates in spontaneously developed diabetes [5]. Therefore, our results reinforce previous data about the importance of oxidative stress in increased apoptosis in diabetes. Studies in other tissues have demonstrated that the binding of AGE such as CML to receptors for advanced glycation end products (RAGE) initiates a cascade of signal transduction and led to ROS generation, activation of Bax, and expression of pro-inflammatory and pro-apoptotic genes, such as c-jun N-terminal kinase [38-41].

As stated previously, there is no consensus in literature on effects of experimental diabetes on proliferative activity in the prostate. Analysis of NOD mice after 20 days of diabetes, based on marker of cell proliferation ki67, indicated increased cell proliferation [5]. On the other hand, previous data from our laboratory revealed a reduction of cell proliferation in the prostate after one week of alloxan-induced diabetes but a normalization was found after three months when the proliferative levels in the normal gland are already low due to ageing of the animals [6]. The present findings are consistent with previous studies which demonstrate that prostate involution after experimental diabetes besides increased apoptosis, is also due to reduction of cell

proliferation [2, 11]. Furthermore, they indicated that the negative effect on proliferating activity of prostate acinar epithelium is not an immediate response to diabetes but persists at medium-term. As previously demonstrated by Yono e cols. for this diabetes model, the inhibition of cell proliferation is caused, among other factors, by the up-regulation of protein Tieg which plays an important role in TGF-β pathway and also of protein Igfbp6, which binds IGF-II and inhibits its mitogenic activity [11]. The alterations in Igfbp6 and Tieg occurred only in hyperglycemic and not in euglycemic streptozotocin-induced diabetes or spontaneous diabetes suggesting that hyperglicemia is an important factor affecting proliferation of molecular prostate response [11]. The molecular data provided by these authors point to a differential prostate response when considering spontaneous or drug-induced diabetes. Further studies are necessary to elucidate the signaling pathways which are involved in altered apoptosis and proliferation in the prostate on diabetes.

A previous histopathological evaluation of rat prostate after three months of alloxan-induced diabetes revealed that most of the animals (64%) showed prostate atrophy but one third (35%) also exhibited neoplasic lesions [8]. Moreover, in this report it was verified that about 7% exhibited PIA (proliferative inflammatory atrophy). The current analysis, as expected, showed that atrophic alterations observed in the prostate after one month of STZ-induced diabetes were intermediate between those described for short and longer times, with acinar cells exhibiting a cubical shape and a transitional phenotype between the normal and the high atrophic aspect detected after three months of diabetes [8]. However, in spite of the high glycemia observed here, no increase in the signs of inflammation and PIA were found in comparison with control rats, and no neoplasic lesion was detected, suggesting that this model of diabetes needs a longer time for the appearance of pre-malign and malign lesions. However, it is noteworthy that once set into motion, RAGE-mediated events may stimulate a chronic cycle of sustained expression of proinflammatory genes [40] that may explain the high incidence of PIA and cancer in prolonged untreated experimental diabetes [8].

Biochemical analyses indicated that one month of diabetes was not sufficient to alter the biomarkers of oxidative stress in blood of rats, but some changes in these markers have been observed in the prostate. A significant increase was noted in GST and CAT activity, accompanied

by a marginal increment in lipid peroxidation. Therefore, it is concluded that the changes in the AOS of prostate in streptozotocin-induced diabetes precede those of blood. The increase in CAT and GST activities may indicate a rise in the antioxidant defense in the gland during diabetes, which may represent an attempt to reverse the oxidative stress levels. Therefore, the present data after one month of experimental diabetes in the rat correspond to an initial phase of disturbance in AOS. It should be noted that studies of long duration with experimental diabetes are difficult to execute because of the low survival rates of animals without proper insulin replacement. On the other hand, insulin therapy normalizes blood glucose levels and, as a consequence reduces the injuries caused by oxidative stress.

Literature data about the AOS in other genital organs such as testis and epididymis of rodents with similar conditions of diabetes (4 weeks) are very discrepant; a decrease of the main enzymatic markers (CAT, GPx, SOD) was observed in some cases [34] and an increase was reported in other studies [33, 35]. Comparing our prostate data with that of above-mentioned studies we conclude that there is no pattern of response among the main biomarkers of AOS among genital organs under experimental diabetes and such response is probably tissue specific. In spite of these discrepancies, there seems to be a consensus in the literature for the GST behavior, whose activity increases in the testis and epididymis, compared to the control after one month of diabetes [34, 35] as observed in this study for the prostate. The GSTs are an important class of enzymes with a crucial role in the detoxification of intracellular reactive electrophiles and products of oxidative stress by catalyzing the conjugation of these compounds with glutathione [42]. Several reports have indicated that GST acts as a protector against neoplasic transformation [24, 42, 43]. The relationship between inflammation and prostate cancer was put forward in several studies [43-46]. The term proliferative inflammatory atrophy (PIA) was proposed to define the lesions exhibiting inflammatory foci, atrophic epithelium and proliferating cells which favor the malign transformation [45, 45]. Contrary to PIN and in neoplasic lesions, GST expression (GSTP1 and GSTA1) is high in PIA, suggesting that these lesions are subject to increased oxidative stress which has a relevant role in malignance [24, 43, 47-49]. Correlating the present histological and biochemical data with previous results from our laboratory [8], it may be suggested that during progression of diabetes, the increase in oxidative stress in the prostate

precedes the PIA and may influence the development of these lesions as well as other pathological alterations. As mentioned, the presence of an ongoing oxidative insult may result in upregulation of GST in PIA [24, 44]. However, it is necessary to mention that the investigations attempting to elucidate the cause-effect connection between prostate cancer and oxidative damage are based on biochemical analysis of biomarkers in the blood and not in the gland itself [18, 22]. These evaluations and imumunocytochemical analysis [23] show that, during malignant progression, there is a decrease in the expression of GST and other oxidative stress markers. Then, it is reasonable to propose that alteration in GST expression in diabetes may be implicated in development of pre-malign and malign lesions previously observed in animal models [8]. Our data indicate that the GST family, besides being a valuable marker for pathological lesions in the prostate, it is also an important component of protective mechanisms against prostate oxidative damage in diabetes.

Several lines of evidence denote the protective effects of supplementation with some antioxidants against prostate cancer, but their action on AOS in other disorders, like diabetes, has not been carefully examined. The vitamin C has the advantage of having a low inherent toxicity in humans and rodents even at high doses [50-52]. In other tests with vitamin C it was observed that this water soluble antioxidant does not normalize the diabetes effects in physiological and biometric status. Our research demonstrated that vitamin C treatment in control rats did not interfere with the activity of antioxidant enzymes and lipid peroxidation levels in the prostate and blood. But it is important to emphasize that vitamin C restored the apoptotic rates, attenuated the AGE immunostaining and GST activity in the prostate of the diabetic group. Then, it is possible that vitamin C had a protective effect, not requiring the increase in GST activity. The antioxidant treatment also had no effect on cell proliferation in diabetes since these proliferating levels are already low due to the decrease of testosterone and other paracrine factors such as IGF and TGF-β [11]. Thus, in this experimental design, the vitamin C partially attenuated the oxidative stress but was not able to recover the proliferative capacity of prostate acinar epithelium probably due to alterations in other biomarkers of oxidative stress, such MDA and CAT.

Conclusions

In conclusion, the present study indicates that oxidative stress is, at least in part, involved in unbalanced cell proliferation and death caused by streptozotocin-induced diabetes in the rat prostate. The increment in GST activities in the prostate of diabetic rats suggests that it is also an important component in defense against oxidative damage in the prostate under diabetes. Additionally, vitamin C supplementation of diabetic rats improved the antioxidant defense concerning GST and recovered the apoptotic rates in the prostate.

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References

1. Cagnon VHA, Camargo AM, Rosa RM, Fabiani CR, Padovani CR. Martinez FE. Ultraestructural study of the ventral lobe of the prostate of mice with streptozotocin induced diabetes (C57bl/6j). Tissue & Cell 2000;32:275-283.

2. Ikeda K, Wada Y., Foster He JR, Wang Z, Weiss RM, Latifpour J. Experimental diabetes-induced regression of the rat prostate is associated with an increased expression of transforming growth factor-ß. J Urol 2000;164:180-185.

3. Ribeiro DL, Candido EM, Manzato AJ, Taboga SR, Cagnon VHA. Prostatic stromal microenvironment and experimental diabetes. Eur J Histochem 2006; 5: 51-60.

4. Burke JP, Jacobson DJ. Mcgree M, Roberts RA, Girman C, Lieber MM, Jacobsern SJ. Diabetes and benign prostatic hyperplasia progression in Olmsted country, Minnesota. Adult Urol 2006;67:22-25.

5. Fávaro WT, Padovani CR, Cagnon, VHA. Ultrastructural and proliferative features of the ventral lobe of the prostate in non-obese diabetic mice (NOD) following androgen and estrogen replacement associated to insulin therapy. Tissue & Cell 2008;2:119-132.

6. Arcolino FO, Ribeiro DL, Gobbo, MG, Taboga SR, Góes RM. Aloxan-induced diabetes increases apoptosis and frequency of p63 positive cells in prostate acinar epithelium. Int J Exp Pathol 2010;91:144-154.

7. Ribeiro DL, Taboga SR, Góes RM. Diabetes induces stromal remodelling and increase in chondroitin sulfate proteoglycans of the rat ventral prostate. Int J Exp Pathol 2009;90(4):400-411.

8. Ribeiro DL, Marques SF, Alberti S, Spadella CT, Manzato AJ, Taboga SR, Dizeyi N, Abrahamsson PA, Góes RM. Malignant lesions in the ventral prostate of alloxan-induced diabetic rats. Int J Exp Pathol. 2008;89(4):276-283.

9. González-perez A, García Rodríguez LA. Prostate cancer risk among men with diabetes mellitus.Cancer Cause Control 2005;16:1055-1058.

10. Turner EL, Lane JA, Donovan JL, Davis MJ, Metcalfe C, Neal DE, Hamdy FC, Martin RM. Association of diabetes mellitus with prostate cancer: nested case-control study (Prostate testing for cancer and treatment study). Int J Cancer 2011;128(2):440-446.

11. Yono M, Mane SM, Lin A, Weiss RM, Latifpour J. Differential effects of diabetes induced by streptozotocin and that develops spontaneously on prostate growth in Bio Breeding (BB) rats. Life Sci 2008;83(5-6):192-197.

12. Baynes W, Watkins NG, Fischer CI, Hull CJ, Patrick JS, Ahmed MU, Dunn JA, Thorpe SR. The Amadori product on protein: structure and reactions. Prog Clin Biol Res 1989;304:43-67.

13. Deyl, Z, Miksík I. Post-translational non-enzimatic modification of proteins. I. Chromatography of marker adducts with special emphasis to glycation reactions. J. Chromatogr B Biomed Sci Appl 1997;699(1-2):287-309.

14. Denis U, Lecomte M, Paget C, Ruggiero D, Wiemsperger N, Lagarde M. Advanced glycation end-products induce apoptosis of bovine retinal pericytes in culture: involvement of diacylglycerol/ceramide production and oxidative stress induction. Free Radicl Biol & Medic 2002;33: 236-247.

15. Yatoh S, Mizutani M, Yokoo T, Kozawa T, Sone, H, Toyoshima H, et al. Antioxidants and an inhibitor of advanced glycation ameliorate death of retinal microvascular cells in diabetic retinopathy. Diabetes Metab Res Rev 2006;22(1):38-45.

16. Thotala D, Chetyrkin S, Hudson B, Hallahan D, Voziyan P, Yazlovitskaya E. Pyridoxamine protects intestinal epithelium from ionizing radiation induced apoptosis. Free Radic Biol Med 2009;47(6):779-785.

17. Spector A. Review: Oxidative stress and disease. J Ocul Pharmacol Ther 2000; 16(2):193-201.

18. Kotrikadze N, Alibegashvili M, Zibzibadze M, Abashidze N, Chigogidze T, Managadze L, Artsivadze K. Activity and content of antioxidant enzymes in prostate tumors. Exp Onc 2008;30(3):244-247.

19. Chan FL, Choi HL, Chen ZY, Chan PS, Huang Y. Induction of apoptosis in prostate cancer cell lines by a flavonoid, baicalin. Cancer Lett 2000;160(2):219-228.

20. Chan JM, Elkin EP, Silva SJ, Broering JM, Latini DM, Carroll PR. Total and specific complementary and alternative medicine use in a large cohort of men with prostate cancer. Urology 2005;66(6):1223-1228.

21. Matos HR, Marques SA, Gomes OF, Silva AA, Heimann JC, Di mascio P, Medeiros MH. Lycopene and beta-carotene protect in vivo iron-induced oxidative stress damage in rat prostate. Braz J Med Biol Res 2006;39(2):203-210.

22. Sarafinovska ZA, Eken A, Matevska N, Erdem O, Sayal A, Savaser A et al. Increased oxidative/nitrosative stress and decreased antioxidant enzyme activities in prostate cancer. Clin Biochem 2009;42:1228-1235.

23. Oberley TD, Zhong W, Szwedal LI, Oberley LW. Localization of Antioxidant Enzymes and Oxidative Damage Products in Normal and Malignant Prostate Epithelium. Prostate 2000;44:144-155.

24. Parsons JK, Nelson CP, Gage WR, Nelson WG, Kensler TW, De Marzo AM. GSTA1 Expression in normal, preneoplastic, and neoplastic Human prostate Tissue. Prostate 2001;49:30-37.

25. Weeks I, Woodhead JS. Chemiluminescence immunoassays. J Clin Immunoassays 1984;7:82-89.

26. Weibel ER Principles and methods for the morphometric study of the lung and other organs. Lab Invest 1963;12:131-155.

27. Peterson GL. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal Biochem 1977;83:346-356.

28. Beutler E. Red Cell Metabolism: A Manual of Biochemical Methods. Grune and Straton;1975, New York.

29. Keen JH, Habig WH, Jakoby WB Mechanism for several activities of the glutatione S-transferases. J Biol Chem 1976;251:6183-6188.

30. Sies H, Koch OR, Martino E, Boveris A. Increased biliary glutathione disulfide release in chronically ethanol treated rats. FEBS Lett 1979;103:287-290.
31. Mccord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969;244(22):6049-6055.

32. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzimol 1990;186:421-431.

33. Armagan A, Uz E, Yilmaz HR, Soyupek S, Oksay T, Ozcelik N. Effects of melatonin on lipid peroxidation and antioxidant enzymes in streptozotocin-induced diabetic rat testis. Asian J Androl 2006;8(5):595-600.

34. Shrilatha B; Muralidhara. Occurrence of oxidative impairments, response of antioxidant defences and associated biochemical perturbations in male reproductive milieu in the Streptozotocin-diabetic rat. Int J Androl 2007;30:508-518.

35. Shrilatha B, Muralidhara. Early oxidative stress in testis and epididymal sperm in streptozotocin-induced diabetic mice: Its progression and genotoxic consequences. Reprod Toxicol 2007;23:578-587.

36. Lenzen S. The mechanisms of alloxan- and streptozotocin induced diabetes. Diabetologia 2008;51:216-226.

37. Chandrashekar V, Steger RW, Bartke A, Fadden CT, Kienast SG. Influence of diabetes on the gonadotropin response to the negative feedback effect of testosterone and hypothalamic neurotransmitter turnover in adult male rats. Neuroendocrinology 1991; 54(1):30-35.

48. Yan SD, Schmidt AM, Anderson GM, Zhang j, Brett J, Zou YS, Pinsky D, Stern D. Enhanced cellular oxidant stress by interaction of advanced glycation end products with their receptors/binding proteins. J Biol Chem 1994;269:9889-9897.

39. Du X, Stotcklauser-farber K, Rosen P. Generation of reactive oxygen intermediates, activation of NF-kappaB, and induction of apoptosis in human endothelial cells by glucose: role of nitric oxide synthase? Free Radic Biol Med 1998;27:752-763.

40. Wautier MP, Chappey O, Corda S. Stern DM, Schmidt AM, Wautier JL. Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. Am J Physiol Endocrinol Metab 2001;280: 685-694.

41. Buccellato LJ, Tso M, Akinci OI, Chandel NS, Budinger GR. Reactive oxygen species are required for hyperoxia-induced Bax activation and cell death in alveolar epithelial cells. J Biol Chem 2004;279(8):6753-6760.

42. Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 1995;30:445-600.

43. De Marzo AM, Nakaiy, Nelson WG. Inflammation, atrophy, and prostate carcinogenesis. Urol Oncol 2007;25(5):398-400.

44. De Marzo AM, Marchi VL, Epstein JI, Nelson WG. Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. Am J Pathol 1999;155(6):1985-1992.

45. Van Leenders GJ, Gage WR, Hicks JL. Intermediate cells in human prostate epithelium are enriched in proliferative inflammatory atrophy. Am J Pathol 2003;162:1529-1537

46. Putzi MJ, De Marzo AM. Morphologic transitions between proliferative inflammatory atrophy and high-grade prostatic intraepithelial neoplasia. Urology 2000;56:828-832.

47. Lee WH, Morton RA, Epstein JI, Brooks JD, Campbell PA, Bova GS, Hsieh WS, Isaacs WB, Nelson WG. Cytidine methylation of regulatory sequences near the pi-class glutathione S-transferase gene accompanies human prostatic carcinogenesis. Proc Natl Acad Sci 1994;91(24):11733-11737.

48. Nakayama M, Bennett CJ, Hicks JL, et al. Hypermethylation of the human GSTP1 CpG island is present in a subset of proliferative inflammatory atrophy lesions but not in normal or hyperplastic epithelium of the prostate: A detailed study using laser-capture microdissection. Am J Pathol 2003;163:923-933.

49. Karaivanov M, Todorova K, Kuzmanov A, Hayrabedyan S. Quantitative immunohistochemical detection of the molecular expression patterns in proliferative inflammatory atrophy. J Mol Histol 2007;38(1):1-11.

50. Cadenas S, Barja G, Poulsen HE, Loft, S Oxidative DNA damage estimated by oxo8dG in the liver of guinea-pigs supplemented with graded dietary doses of ascorbic acid and α-tocopherol. Carcinogenesis1997;18:2373-2377.

51. Yasser HA, Abdel-Wahab, Finbarr PM, O'Harte, Mark H, Mooney, Christopher R, Barnett, Peter R, Flatt .Vitamin C Supplementation Decreases Insulin Glycation and Improves Glucose Homeostasis in Obese Hyperglycemic (ob/ob) Mice. Metabolism, 2005;.51(4) 514-517.

52. Gupta R, Dhakal B, Thakur A, Ahnn J Vitamin C and vitamin E protect the rat testes from cadmium-induced reactive oxygen species. Molecules and Cells 2004:17:132-139.

Table

Table I. Mean values and standard deviation of body weight, prostate relative weight, blood glucose levels, ratio, water intake and median values and standard deviation for serum testosterone and estradiol levels of the control group (C), control treated with vitamin C (C + V), non treated diabetic (D) and diabetic treated with vitamin C (D + V). a – statistically different from C; b – statistically different from C + V and c- statistically different between D and D + V.

	C	**C + V**	**D**	**D + V**
Body weight (g)	444.22 ± 53.74	397.8 ± 17.24	264.66 ± 47.1 [a,b]	252.73 ± 42.92 [a,b]
Prostate weight (mg)	535.11 ± 84,2	420.3 ± 137.63	269.57 ± 68.53 [a,b]	295.26 ± 113.11 [a,b]
Prostate relative weight ($x10^3$)	1.23 ± 0.30	1.055 ± 0.34	1.0 ± 0.18	1.17 ± 0.48
Blood glucose (mg/dL)	114.42 ± 18.41	112.55 ± 22.17	483.94 ± 64.53 [a,b]	491.75 ± 86.26 [a,b]
Ratio intake (g/day/animal)	26.49 ± 8.97	31.24 ±12.34	35.46 ± 11.32	29.06 ± 13.27
Water intake (mL/day/animal)	47.42 ± 9.28	51.38 ± 20.34	174.93 ± 54.97 [a,b]	125.27 ± 53.33 [a,b,c]
Serum testosterone (ng/dL)	125 ± 44.2	128.4 ± 45.4	12.6 ± 5.6 [a,b]	3.4 ± 1.2 [a,b]
Serum estradiol (mg/dL)	25.1 ± 8.87	26.1 ± 9.21	21.8 ± 7.26	22.9 ± 6.61

Figure legends

Figure 1. Histological sections in histo resin of rat VP stained with H & E. A and B, control group (C); C and D, control group treated with vitamin C (C + V); E, F and G, untreated diabetic group (D) and H, I and J, diabetic group treated with vitamin C (D + V). Signs of acinus shinkage (F) and epithelial fragments in the lumen were found in the diabetic animals (I). Legend: e – epithelium, s – stroma, l – lumen, v – blood vessel, arrowhead – points of epithelial shrinkage and * - epithelium fragments. A, C, E, F, I and H: 200X, bars = 50 μm; B, D, G and J: 1000X, bars = 10 μm.

Figure 2. Imunnohistochemical localization of Nε-(carboxymethyl) lysine (CML) in rat VP sections. Diabetic animals showed intense CML expression. A, control (C); B, control treated with vitamin C (C + V); C, untreated diabetic (D); D, diabetic treated with vitamin C (D + V), E, positive control and inset, negative control. A, B, C, D, and E: 400X, bars = 25 μm; inset: 200X, bar = 50 μm. F, relative frequency (%) of CML immunostaining areas of ventral prostate of control group (C), control treated with vitamin C (C + V), untreated diabetic (D) and diabetic treated with vitamin C (D + V).

Figure 3. Levels of catalase (A and B), GST (C and D), GPx (E and F), SOD (G) activities (U/mg protein) and MDA (H and I) (TBARS/ mg tissue and TBARS/ mL plasm) in VP extracts and blood samples of control group (C), control treated with vitamin C (C + V), untreated diabetic (D) and diabetic treated with vitamin C (D + V) rats. The values are mean ± standard deviation. a – statistically different from C; b – statistically different from C + V and c - statistically different between D and D + V. Seven animals per group were used for each biomarker of oxidative stress.

Figure 4. Immunocytochemistry for androgen receptor (AR) and its quantification in the rat VP. A, control (C); B, control treated with vitamin C (C + V); C, untreated diabetic (D); D, diabetic treated with vitamin C (D + V). A, B, C, and D: 400 X, bars = 25 μm. The arrows indicate the AR negative cells (blue nuclei). E – relative frequency (%) of AR positive cells. F and G show

the box plot for testosterone (T) and estradiol (E) serum levels (ng/dL) respectively. (C), control treated with vitamin C (C + V), untreated diabetic (D) and diabetic treated with vitamin C (D + V). a – statistically different from C; b – statistically different from C + V.

Figure 5. Variations in frequency of apoptotic (A-D) and proliferating (E-H) epithelial of rat VP, as indicated by immunocytochemical (A-H) and quantitative analyses (I, J). At the immunocytochemical reactions the arrowheads indicate TUNEL- (A, B, C and D) and PCNA- (E, F, G and H) – positive cells. Apoptotic cells were more frequent in the distal regions. A and E, control (C); B and F, control treated with vitamin C (C + V); C and G, non treated diabetic (D) and D and H, diabetic treated with vitamin C (D+V). A, B, C, D, E, F, G and H: 400 X ,bars = 25 μm. I and J - relative frequency (%) of TUNEL-(I) and PCNA-(J) positive prostate cells of control group (C), control treated with vitamin C (C + V), non treated diabetic (D) and diabetic treated with vitamin C (D + V). a – statistically different from C; b – statistically different from C + V.

Figure 1

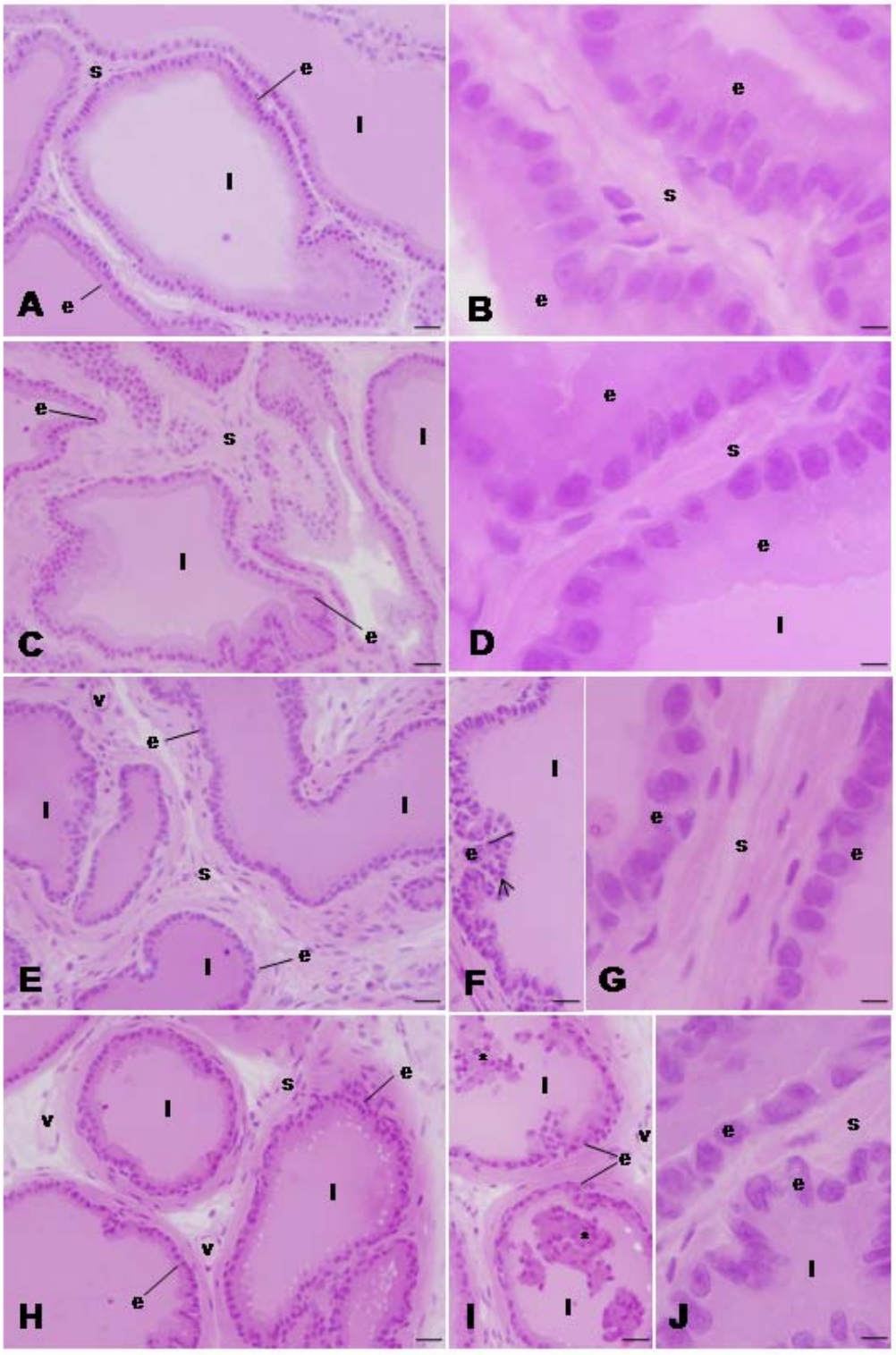


Figure 2

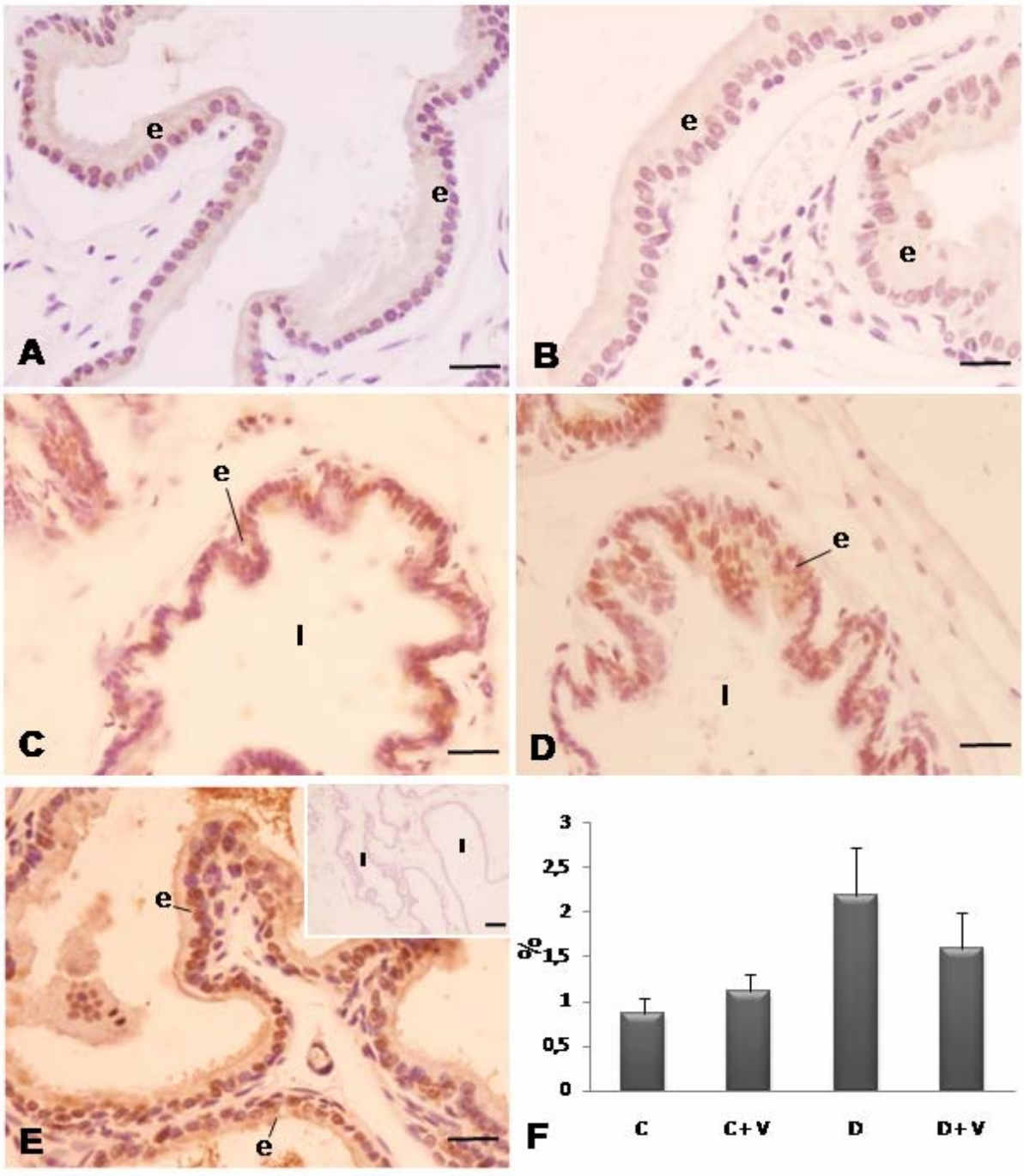
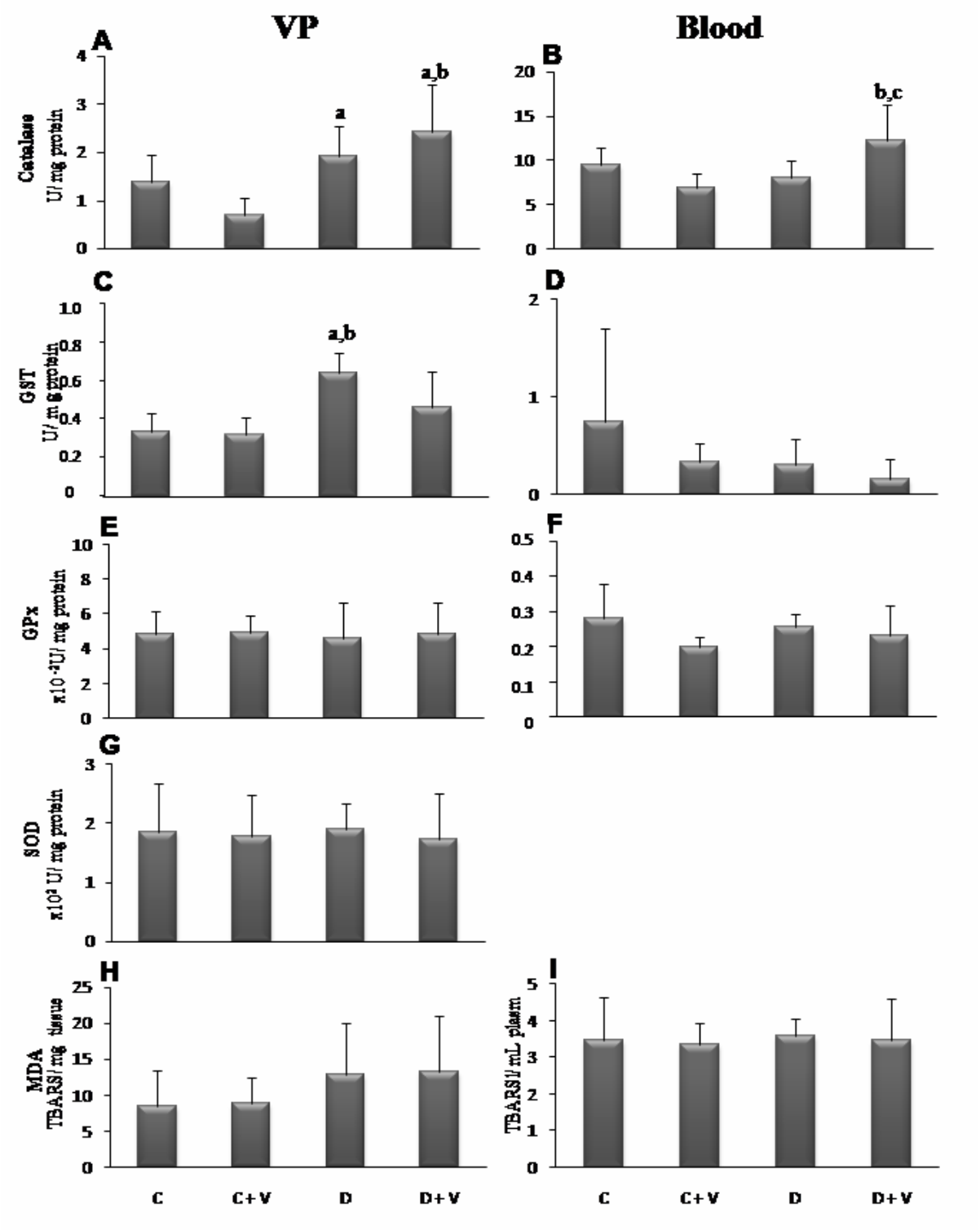


Figure 3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SOD
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D+V

Figure 4

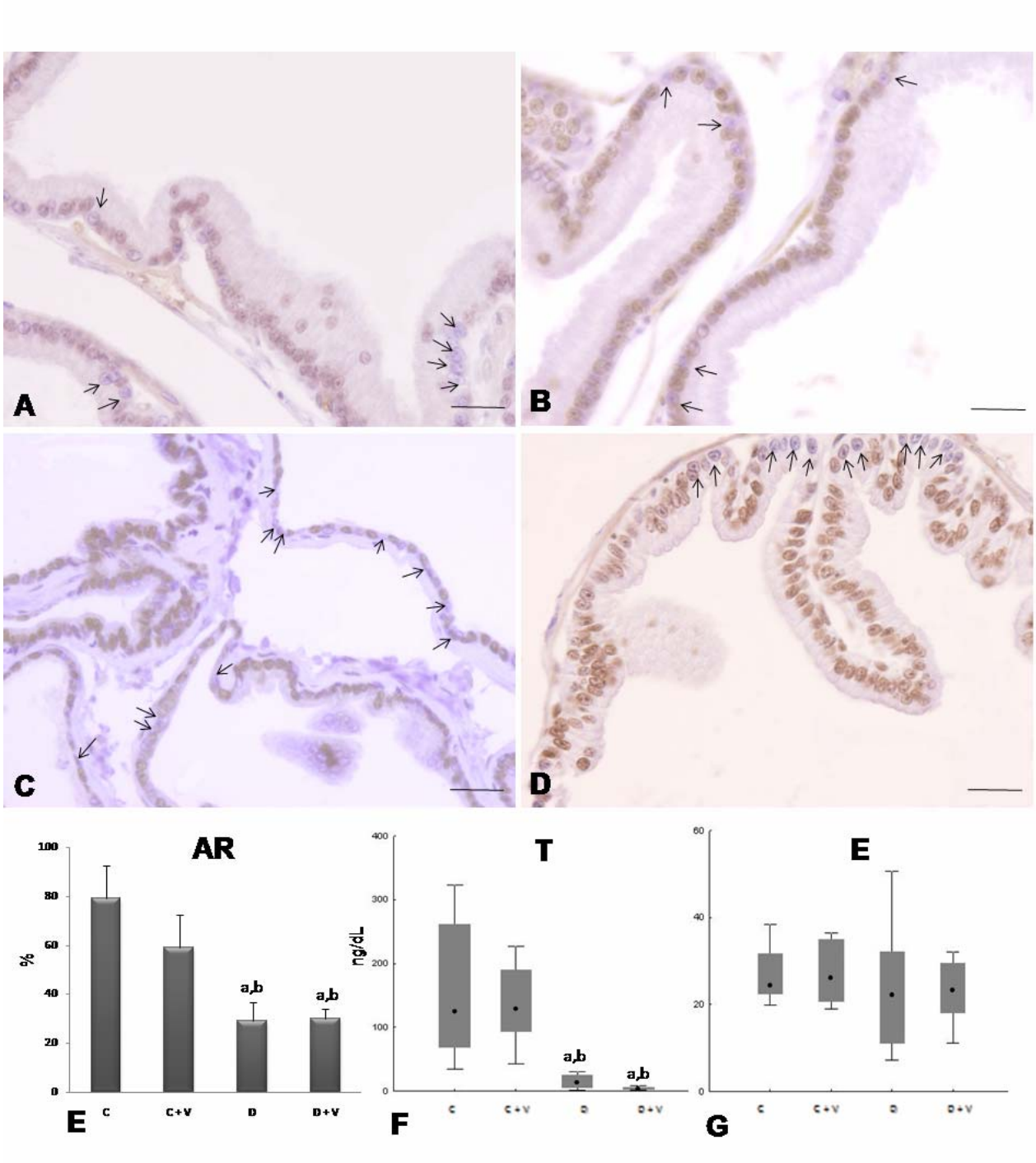
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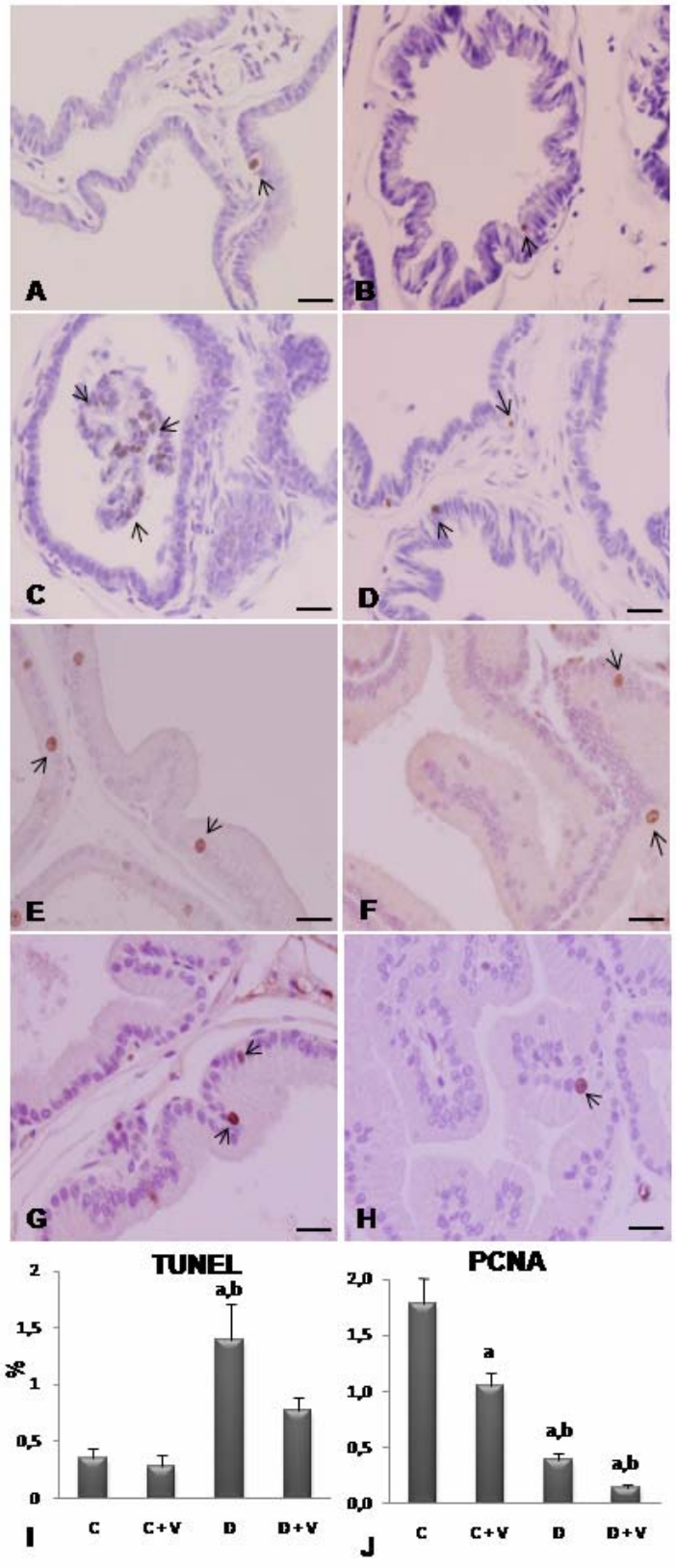
Figure 5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Inverse relation between glycemia and testosterone/estradiol ratio modulates histological changes in the ventral prostate of diabetic rats

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Abstract

The aim of this study was to examine if there is an association betwenn the degree of histological impairment of rat ventral prostate at short-term after alloxan-induced diabetes and blood glucose or circulating sex steroid hormones. A single alloxan injection (42 mg⁄ kg b.w.) was employed to induce diabetes in Male adult Wistar rats and diabetic rats were treated with 5 U/day of insulin or saline solution. One week after diabetes onset, rats were killed, blood glycemia and plasma sex steroids were evaluated and the prostate processed for light microscope analysis. Five groups were assigned according to their glycemia as: control (CT - glycemia 110 mg/dL; n= 6), untreated diabetic 1 (UD1 – glycemia 250-450 mg/dL; n= 6) and 2 (UD2 – glycemia 550 mg/dL; n= 5) and insulin-treated diabetic euglycemic (IDE - glycemia < 130mg/dL; n= 4) and hyperglycemic (IDH – glicemia 250 - 450 mg/dL; n = 5). Microscopic evaluation of prostate was performed using a double-blind method. The plasma estradiol and testosterone levels were determined from chemiluminescence assays and fasting glucose by the glucose oxidase method. An inverse correlation was observed between prostate weight and blood glucose levels and also with testosterone/estradiol rate. The degree of prostate involution was higher in hyperglycemic animals and visualized by severe atrophy especially in the distal tips of ducts. The insulin treatment does not completely prevent the changes caused by diabetes, mainly if glycemic control is not effective. These data suggest that hyperglycemia plays an important role in prostate changes due to diabetes. Although this research does not report short-term drastic tissue changes it suggests that prolonged hyperglycemia coupled with the hormonal imbalance is responsible for the drastic changes observed in the prostate of animals with long term diabetes and without insulin replacement.

Introduction

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia resulting from defects in secretion or action of insulin (Ordóñez *et al.,* 2007, Suthagar *et al.,* 2008). Diabetes complications involve multiple factors with emphasis on hyperglycemia (Lamers *et al.,* 2007). Prolonged hyperglycemia leads to the formation of advanced glycation end products and activation of several biochemical pathways, such as the hexosamine and protein kinase C (Lin *et al.,* 2005; Reis *et al.*, 2008).

Animal studies have indicated that diabetes reduces accessory sex gland weight, circulating androgens and androgen receptors (AR) in prostate cells (Daubrese *et al.*, 1978; Chandrashekar *et al.*, 1991; Ho, 1991, Saito *et al.*, 1996, Ribeiro *et al.*, 2006; Fávaro *et al.*, 2008; Arcolino *et al.*, 2010). The prostate is highly dependent on androgens which and their effecs are mediated by specific receptors (Cunha *et al.*, 1987). Androgenic stimuli regulate gene expression of secretory proteins such as PSA and growth factors involved in maintaining normal prostatic activities (Huang *et al.*, 2002). Estrogen is also involved in prostate homeostases and thus, changes in critical balance between androgens and estrogens may favor the development of prostate diseases (Risbridger *et al.*, 2003). Because diabetes interferes in the production of testosterone as well as in the expression of AR in prostate, it is evident that this disorder negatively affects the prostate functions as has been recently reported (Cagnon *et al.,* 2000; Soudamani *et al.*, 2005; Ribeiro *et al.*, 2006, 2008a).

The association between type 2 diabetes and different human cancers has been a concern for a long time and despite some important gaps in our knowledge still persist the available of large-scale registries led to conclusion that diabetes reduces risk of prostate cancer (Smith and Gale, 2010). On the other hand, animal studies with short-term spontaneous or induced diabetes have shown an increase in prostatic intraepithelial neoplasia (Ribeiro *et al.*, 2006, 2008a). In addition, previous experiments with longer time of alloxan-induced diabetes (three months of diabetes) without insulin replacement revealed a wide individual variation in the morphological response of the rat prostate (Ribeiro *et al.*, 2008a, 2009). In this study, most of animals (~64%)

exhibited severe prostatic atrophy, in a small part of them the prostate remains practically unchanged and one third developed malignant lesions. It is therefore necessary to improve our knowledge about the factors involved in individual variations observed for the prostate changes in laboratory rodents with experimental diabetes and its particular relationship with those observed in 2 diabetes in humans. In the present investigation it was evaluated if there is an association between the degree of histological impairment of the rat ventral prostate at short-term after alloxan-induced diabetes and blood glucose or circulating sex steroid hormones. The data showed an inverse correlation between blood glucose and testosterone levels, reflecting changes in the prostate, which are more dramatic in animals with higher blood glucose levels. Insulin treatment reverses tissue changes but just partially the biometric ones in a glucose-dependent manner.

Material and Methods

Diabetes induction

We used adult male Wistar rats, (3-4 months old), weighing between 300 and 400g, supplied by Central Animal Breeding of São Paulo University- USP, Ribeirão Preto, Brazil. The animals handling and experiments were performed according to the rules of the Ethics Committee on Animal Experiments (UNESP / Botucatu, protocol. 17/07-CEEA). For diabetes induction, rats (n = 40) were kept fasted for 24 hours and injected intravenously with 0.1 mL saline containing 42 mg/kg b.w. of alloxan (5.6 Dioxiuracil monohydrate, Sigma, St. Louis, MO, USA) in the penian vein. After 30 minutes, rats were fed normally. The control rats (n = 10) received only the saline injection. Glucose levels were tested after 2 days of diabetes induction using blood samples from the fingertips and the glucose monitor Accu-Chek (Roche Diagnostics, Mannheim, Germany). Part of the diabetic rats was separated and received daily treatment with 5 IU of insulin (Humulin, Biobras, MG, Brazil) throughout the experimental period. One week after diabetes confirmation, rats were grouped according to their glycemia as: control (CT - glycemia 110 mg/dL; n= 6), untreated diabetic 1 (UD1 – glycemia 250-450 mg/dL; n= 6) and 2 (UD2 –

glycemia 550 mg/dL; n= 5) and insulin-treated diabetic euglycemic (IDE - glycemia < 130mg/dL; n= 4) and hyperglycemic (IDH – glicemia 250 - 450 mg/dL; n = 5).

Histological and morphometrical analysis

All groups were killed by CO_2 inhalation followed by decapitation. After the excision of the prostatic complex, the ventral lobes were separated and weighed. Prostatic fragments were fixed by immersion in Karnovsky solution (2.5% formaldehyde, 2.5% glutaraldehyde in a phosphate buffer 0.2 M pH 7.2) for 24 hr and embedded in plastic resin (Historesin Embedding Kit; Leica,) for histological processing. Sections (3µm thickness) were done in an automatic microtome (Leica, Nussloch, Germany) and stained with hematoxylin and eosin. The analysis of tissue sections was performed by double-blind method with an Olympus CH30 light microscope (Olympus, Center Valley, PA, USA) coupled with a CCD camera and a semi-automatic image analysis system (Image-Pro Plus Media Cybernetics version 4.5 for Windows software, Bethesda, MD, USA).

The height of acinar prostatic epithelium was estimated by using 2 histological sections from different prostate fragments per animal. Ten microscopic fields were digitalized per histological section at 1000x magnification and 20 measurements were performed by field.

Plasma hormonal quantification

Blood samples were collected immediately after decapitation. Plasma was separated by centrifugation and stored at 20˚C for subsequent assays. Quantification of plasma testosterone and estradiol was performed using the Modular Analyzer for Immunoassay of Chemiluminescence ECI (Johnson and Johnson, Langhorne, PA, USA) (Weeks & Woodhead 1984). Five rats were used from each group, and the test was performed in triplicate. The intra- and inter-assay variations were 4.6% and 4.3% respectively.

Statistical Analysis

The data were analyzed using Statistica 6.0 Software (Copyright©StatSoft Inc., Tulsa, OK, USA). Quantitative variables were compared using ANOVA followed by Tukey test. The

power of association between the quantitative variables was determined using Pearson linear correlation. p-values < 0.05 were considered as significant.

Results

Most of alloxan- induced diabetic rats showed glycemia above 500mg/dl, but a variation of 228- >600 mg/dl was observed. Due to this variation, insulin therapy with only one daily dose was efficient to glycemic control of about half of the animals. As shown in Figure 1A, when taken together all diabetic animals no submitted to insulin replacement, it was observed that experimental diabetes caused, after a week, a marked reduction in ventral prostate weight which paralleled the testosterone withdrawal (Fig. 1). Insulin treatment maintained serum testosterone and diminished the prostate atrophy (Fig. 1). Plasma estrogen levels did not differ among control, untreated and insulin-treated groups (Fig. 1). It was found that the untreated diabetic animals showed marked loss of body weight, especially those of UD2, while control and diabetic insulin-treated rats gained body weight (Table 1). As expected, body weight gain was improved by insulin therapy, particularly euglicemic group (Table 1).

Statistical analysis showed a marked inverse correlation between glycemia and the following parameters: testosterone (Correl= -0.64, p= 0.0009), testosterone/estradiol ratio (-0.52, p= 0.02) and prostate weigth (- 0.67, p= 0.0002). In this regard, as higher glycemia lower is testosterone and T/E, independent from studied group. In the same way, T/E also decreased in untreated diabetic animals in an inverse manner to blood glucose levels, although it was not statiscally significant (Fig. 2). The same was observed for the insulin-treated group, rats with inefficient glucose control (IDH) had lower T/E. Interestingly, in both groups, ventral prostate weight decreased proportionally with the decrease of testosterone and T/E, in a glucose-dependent manner, as well as the estradiol levels (Fig. 2). However, in IDE rats this proportion is lost, and the prostatic weight is considerably lower than the T/E ratio, even lower than the group with low glucose control (Fig. 2).

Morphological analysis showed that the ventral prostate of control rats had a typical tubuloalveolar structure with acini exhibiting folded epithelium with columnar cells and basal

nucleus (Fig. 3A). In UD1 were not observed drastic histological changes it was not observed in the ventral prostate, but, a high degree of atrophy was verified to UD2 (Fig. 3D and E). Thus, the prostatic lobes of the later exhibited glandular shrinkage, especially in the distal tips of the gland (Fig. 3D). In this group, acinar atrophy was characterized by involuted acini, low luminar amplitude and intense folding of the mucosa (Fig. 3D). Prostate of IDE rats was in general similar to the control, a but few areas of glandular atrophy have been observed in some animals, however in this case, they were localized and not reach the extension of UD2 (Fig. 3H). Similarly, the shrinkage of the distal regions in IDE was restricted to discrete areas when compared to other diabetic groups (Fig. 3I). On other hand, IDH rats exhibited a glandular structure similar to UD1 (Fig. 3F) with shrinkage in the distal portions of the gland (Fig. 3G).

The analysis of the epithelium and subepithelial stroma region showed that the cellular atrophy is greater in diabetic rats with moderate glucose levels (Fig. 4B, Table 1) and that the atrophy caused by high glucose levels is characterized by severe disruption of epithelial structure, with disorganized cell distribution and adjacent stroma accompanied this disorganization (Fig. 4C and D). Among animals treated with insulin, there was a minor impairment of the structure, prostate epithelial cells were layer organized and exhibited an elongated shape, being similar to the control prostate (Fig. 4E), especially in those animals with good glycemic control (Fig. 4F). The subepithelial layer appears thicker in the group with normal glucose levels when compared with insulin treated groups and other diabetic groups (Fig. 4B,D and F).

Discussion

This work has shown that there is an inverse correlation between the relative weight of the prostate, testosterone/estradiol (T/E) and blood glucose levels. Moreover, despite the tissue changes in ventral prostate of short-term diabetic rat were not as significant if compared to long-term diabetes, they are intensified in animals with high blood glucose and can be partially reversed with glycemic control by insulin. This work shows for the first time that different blood glucose levels modulate morphological changes in prostate, even in insulin treated diabetic animals.

In general, diabetes caused a significant reduction in testosterone levels as well as on body weight and relative weight of rat ventral prostate. These changes were partially recovered with insulin replacement. It is well known that androgens are essential for both cell proliferation and differentiation during embryonic development and postnatal prostate, but also for maintaining its structure and functional activity in adults (Cunha *et al.*, 1987, Wilson *et al*., 1993, Quigley *et al.*, 1995, Marker *et al.*, 2003). Besides testosterone, other hormones are important in prostate functioning, including insulin, since the prostate has receptors for insulin and this hormone has a strong influence on cell proliferation (Saucier *et al.*, 1981). It is well established that diabetes causes a reduction in testosterone levels and in the expression of androgen receptors in the prostate, indicating that the deleterious effects of diabetes are caused by inefficient action of androgens in the gland. However, studies have shown that testosterone replacement in diabetic animals can not reverse the prostate atrophy or plasma testosterone levels (Soudamani *et al.,* 2005). Insulin treatment also failed to fully reverse the testosterone levels, as demonstrated in this work and others (Arcolino *et al.,* 2010, Fávaro *et al.,* 2008). These data confirmed previous results (Ribeiro *et al.,* 2006) and show that the effects of androgen deficiency are intensified by the absence of insulin, indicating the need for replacement of both hormones in the diabetic animals for proper maintenance of prostatic activity.

Although there are few studies on the effects of diabetes on prostate morphophysiology, such studies do not discuss the different mechanisms involved in diabetes that could cause glandular changes. Since diabetes involves multiple metabolic disorders, lack of insulin action, hyperglycemia and changes in gonadal axis leading to decreased serum testosterone, it becomes difficult to identify the main contributor to the observed changes in the prostate. Ribeiro *et al* (2008b), in a study on insulin resistance, showed that rats with mild hyperglycemia and inefficient insulin action have epithelial changes that are quite different from those observed in situations of androgen ablation, suggesting a role of insulin in prostatic morphophysiology. Although the debate about the role of insulin had increased recently, yet little is known about the lack of effective action of insulin and hyperglycemia its influence on the activity of the prostate.

The analysis of the diabetic rats treated and untreated with insulin with different ranges of glycemia showed that changes in testosterone and estrogen levels are more intense with the

increase of blood glucose. The same was observed in the morphology of the prostate, which showed more drastic involutive changes in rats with very high blood glucose levels. In the same way, there was a major recovering of these changes in those with better glycemic control. It is known that hyperglycemia is the factor responsible for most of the complications related to diabetes and is involved in oxidative stress, formation of advanced glycation end products and activation of multiple biochemical signaling pathways (Lin *et al.,* 2005; Yono *et al.,* 2008). A recent research has shown that there is an inverse relationship between testosterone levels and glucose, but the mechanisms involved in this process remain to be elucidated (Dawood *et al.,* 2005). Moreover, Ramamani *et al.* (1999) demonstrated that castrated rats present high levels of blood glucose. Diabetic rats with controlled glycemia have inefficient testosterone levels, lower than those animals with normal blood glucose, showing the importance of glucose control for restoration of androgen levels (Fushimi *et al.,* 1989). In humans, a significant inverse association between total testosterone and fasting glucose has also been described (Goodman-Gruen, 2000). Our data confirm this inverse relationship and thus, indicate that hyperglycemia is probably an essential component of androgen-dependent changes of the prostate of diabetic animals.

The T/E and prostate weight decreased proportionally in relation to one another and in inverse proportion to glucose levels. That is, with increasing hyperglycemia lower the T/E and smaller is also the prostate weight. Although androgens are the primary regulators of prostatic homeostasis, the balance between androgen and estrogen seems to have equal importance in this function (Risbridger *et al.*, 2003). Studies show that an imbalance of this hormonal interaction is one of the main factors in the development of prostate pathologies (Weihua *et al.*, 2002). Suthagar *et al.* (2008) and Sudha *et al.* (2000) showed that diabetes promotes a reduction in levels of estradiol and its receptors in rats. However, a study in aging and diabetic humans showed increased levels of this sex steroid, although the values were not significant (Bahia and Gomes, 2003). Whereas the long-term diabetes is known to lead to the development of benign and malignant in rat prostate (Ribeiro *et al.,* 2008a), the strong imbalance between testosterone and estradiol observed in this investigation of short term diabetes may be a factor which favor the incidence of lesions in this gland in the long term.

This investigation found an inverse relationship between hyperglycemia and biometric (body and ventral prostate weight), hormonal (testosterone and estradiol) and tissue parameters in the ventral prostate of diabetic rats, suggesting that the degree of prostatic involution caused by experimental diabetes is dependent on the glycemic status. The treatment with insulin does not completely prevent the modifications caused by alloxan-induced diabetes, especially if glycemic control is not effective. Although this research does not report drastic short-term tissue changes, it is important to clarify that probably prolonged hyperglycemia coupled with the hormonal imbalance is responsible for the striking changes observed in rat prostate of long-term diabetes without insulin treatment.

Tables

Table 1- Variations in body weight gain of rats and height of prostate acinar epithelium of control (CT), untreated diabetic 1 (UD1) and 2 (UD2) and insulin-treated diabetic euglycemic (IDE) and hyperglycemic (IDH) groups. Values represent mean and standard devation.

		Insulin-treated		**Untreated**	
	CT	**IDE** (<130 mg/dl)	**IDH** (250-450 mg/dl)	**UD1** (250-450 mg/dl)	**UD2** (>550 mg/dl)
Body weight gain	85 ± 21.3 [a]	79 ± 9 [a,c]	49 ± 24.3 [a,c]	-1.6 ± 68.6 [b,c]	-60 ± 24.7[b]
Epithelial height (μm)	48.5 ± 2.5 [a]	48.5± 1.4 [a]	46.6 ± 1 [a,b]	34.5 ± 1.2 [b]	46.7 ± 7.2 [a,b]

[a,b,c] Different superscriptions letters represent the significant difference between groups ($p < 0.05$, Tukey Test).

Figure Legends

Figure 1- Variations in the rat ventral prostate weight (black bars), plasma testosterone (white bars) and estradiol (triangles) between control, untreated diabetic and insulin- treated diabetic rats.

Figure 2- Variations in ventral prostate weight (blue), testosterone/estradiol ratio (pink) and estradiol (yellow) in control (CT), untreated diabetic 1 (UD1) and 2 (UD2) and insulin-treated diabetic euglycemic (IDE) and hyperglycemic (IDH) groups. Values represent average ± standard error. a,b- $p < 0.05$.

Figure 3- Histological pattern of the ventral prostate of control (CT), diabetic with moderate (UD1) or high glycemia (UD2) and insulin treated diabetic rats with high (IDH) or normal glycemia (IDE). **A-** Control rats exhibit large acini with epithelial folds and a loose connective tissue in the stromal space. **B, C-** The prostate of diabetic rats with moderate glycemia showed points of epithelial atrophy (*) represented by acini with a wide lumen and short epithelial cells. The distal portions of gland produced discrete epithelial foldings (arrows). **D, E**- Intense glandular involution in the prostate of diabetic animals with high glycemia, showing reduced and shrunken acini (a), strait lumen and increased folding in the acinar epithelium (arrows). **F, G-** The prostate of IDH group very similar to diabetic rats showing moderate glycemia, exhibiting points of glandular shrinkage in the distal regions (arrows). **H, I-** In IDE rats, most of gland is represented by typical acini, similar to control prostate, however, there are some points of gland shrinkage (*) and also epthelial foldings in the distal tips of gland (arrows). Scale Bars: A, B, D, F, H- 400 μm; C, E, G, I- 200 μm.

Figure 4- Epithelial region of ventral prostate of control (A), diabetic (B, C) and insulin treated diabetic rats (D, E). **A-** Prostatic epithelium (e) of control group is represented by columnar cells, abundant proteic secretion (se) and thick layer of subjacent stroma (s). **B-** Diabetic animals with moderate hyperglycemia showed epithelial atrophy (e) with cubic cells and round nuclei. The

secretory activity is evident in lumen (se) and the stromal region had no evident changes (s). **C-** In those diabetic rats with intense hyperglycemia the most evident change is represented by epithelial disorganization (arrows). In detail, it is observed that epithelial cells lost their layer organization and the adjacent stroma follow the epithelial disorganization and lost its concentric subepithelial arrangement (s). **E e F**- Insulin treated rats showed typical prostatic structure of control animals, with tall cells, layer organized epithelium (e) and stroma (s). There were no morphological changes between different glycemic levels in this group. Scale bars: A, B, D, E, F- 20 μm; C- 40 μm.

Figure 1

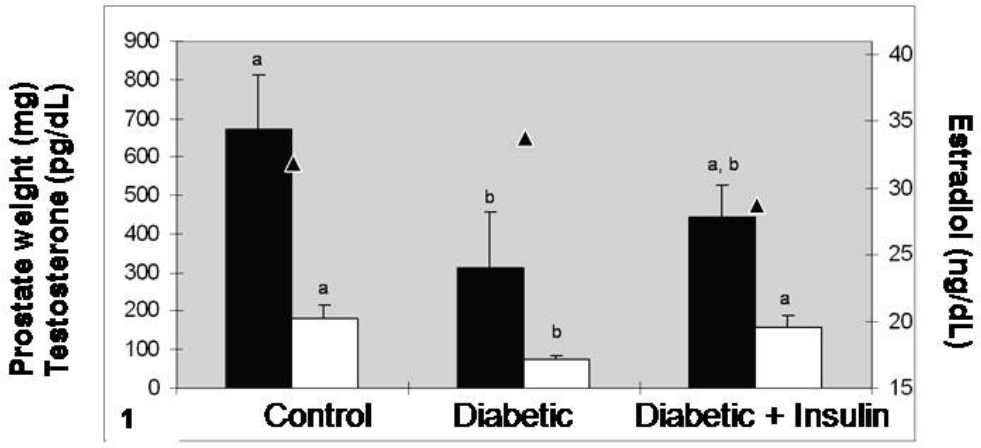


Figure 2

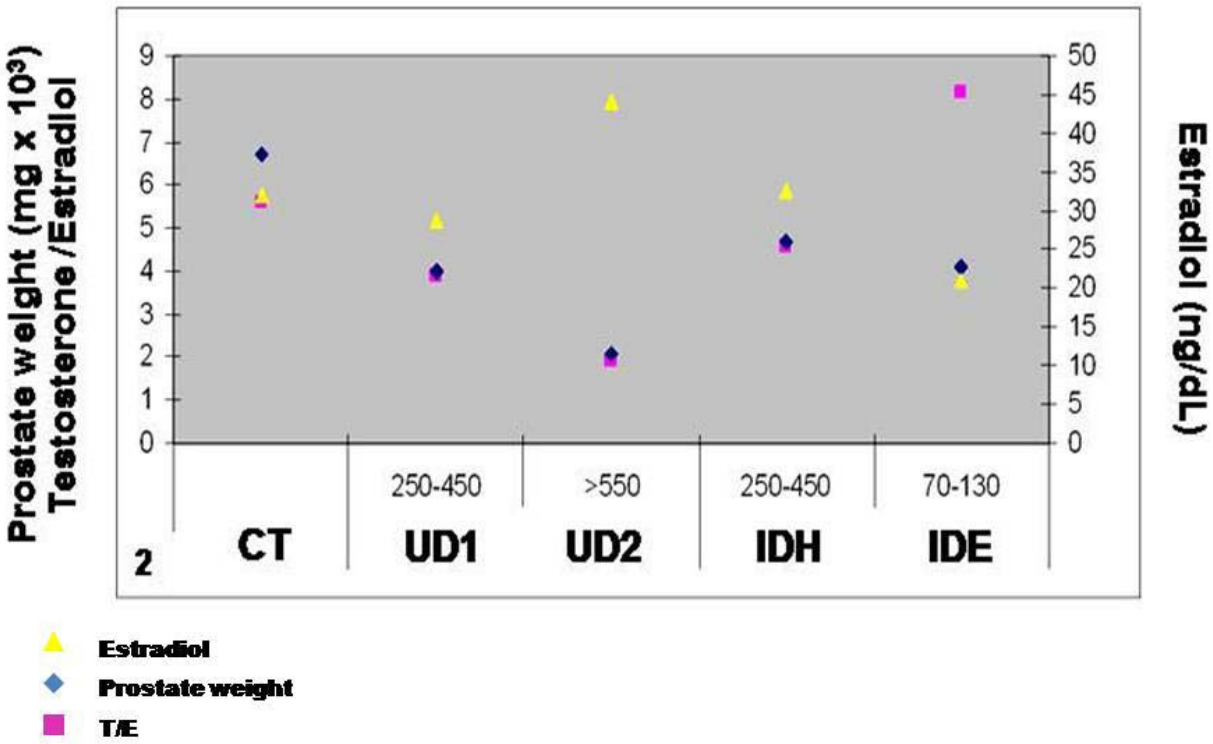


Figure 3

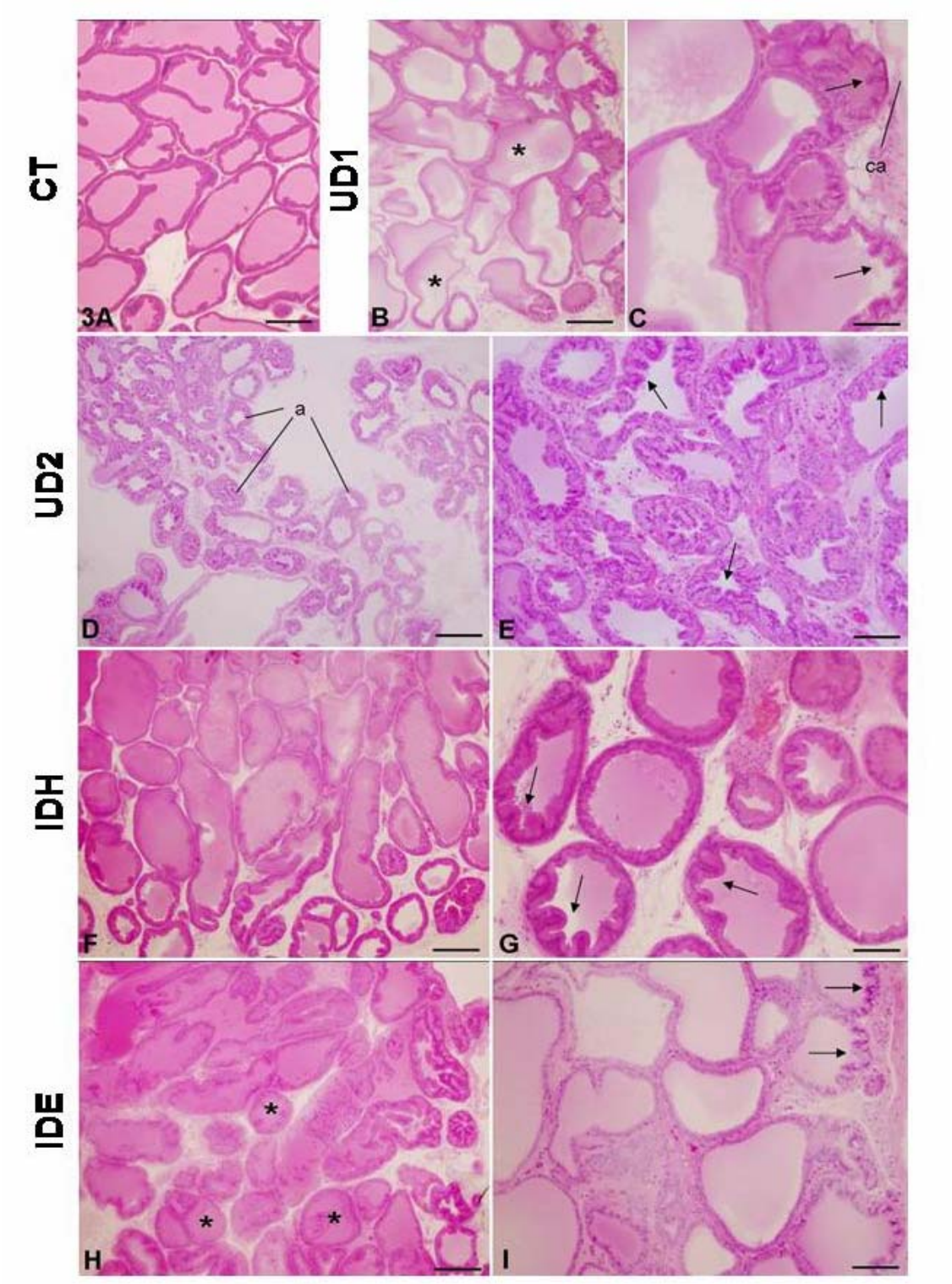
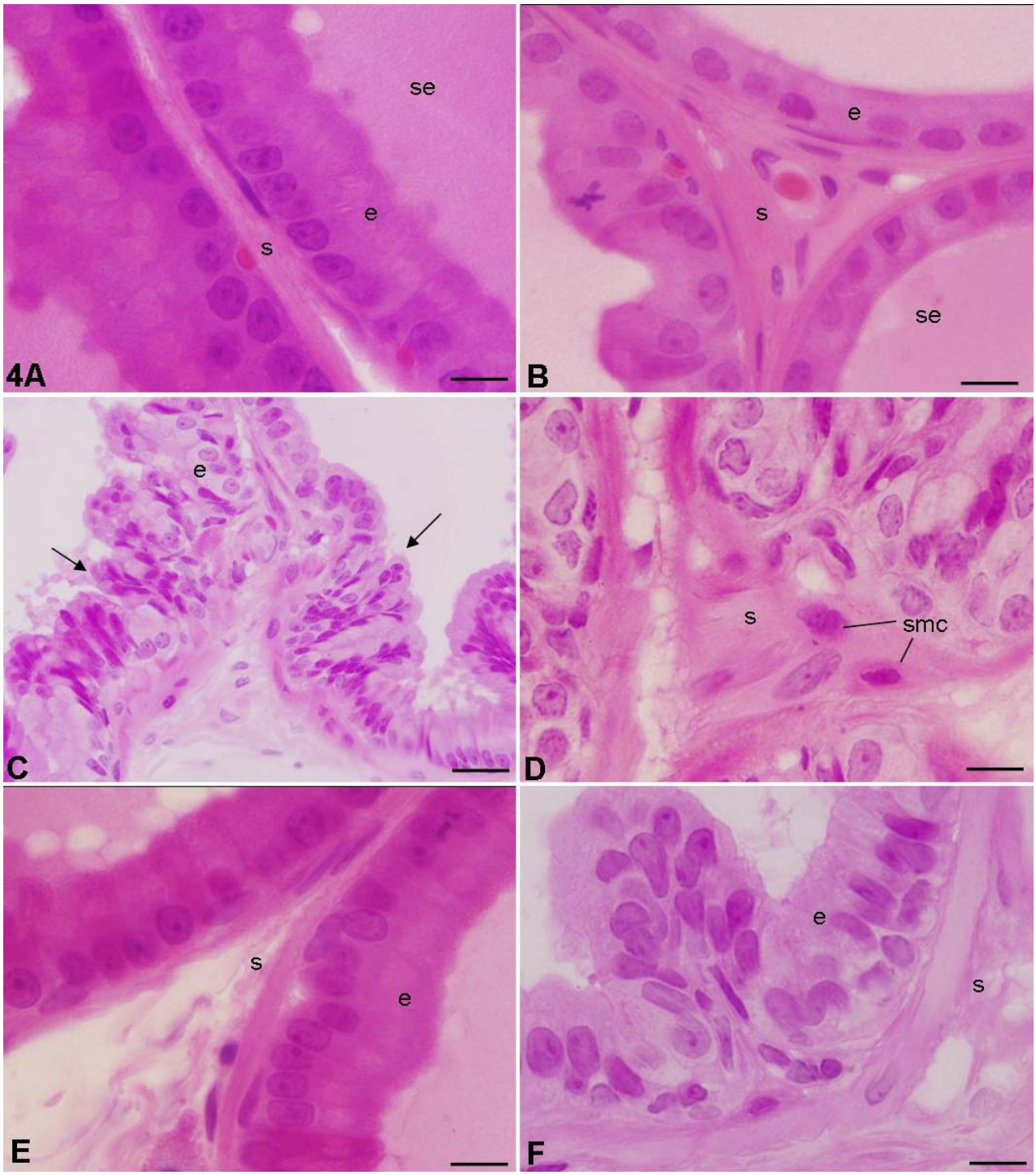


Figure 4



References

ARCOLINO, F.O.; RIBEIRO, D.L.; GOBBO, M.G.; TABOGA, S.R.; GÓES, R.M. Aloxan-induced diabetes increases apoptosis and frequency of p63 positive cells in prostate acinar epithelium. Int J. Exp Pathol., v. 91, p. 144-154, 2010.

BAHIA, L.; GOMES, M.B. Influence of age and diabetes on sex steroids an SHBG in men. Arq Bras Endocrinol Metab., v. 47, p. 256-260, 2003.

CAGNON, V.H.A.; CAMARGO, A.M., ROSA, R.M.; FABIANI, C.R.; PADOVANI, C.R.; MARTINEZ, F.E. Ultrastructural study of the ventral lobe of the prostate of mice with streptozotocin induced diabetes (C57bl/6j). Tissue Cell. v. 32, p. 275-283, 2000.

CHANDRASHEKAR, V.; STEGER, R.W.; BARTKE, A.: FADDEN, C.T.; KIENAST, G. Influence os diabetes on the gonadotropin response to the negative feedback effect of testosterone and hypothalamic neurotransmitter turnover in adult male rats. Neuroendocrinology. v. 54 p. 30-35. 1991.

CUNHA, G.R.; DONJACOUR, A.A.; COOKE, P.S.; MEE, S.; BIGSBY, R.M., HIGGINS, S.J., SUGIMURA, Y. The endocrinology and developmental biology of the prostate. Endocr Rev. v. 8, p. 338-361, 1987.

DAUBRESE, J.C.; MEUNIER, J.; WILMOTTE, A.S.; LUYCKX, A.S.; LEFEBVRE, P.J. Pituitary-testicular axisin diabetic men with and without impotence sexual. Diabetes Metab., v. 4, p. 233-237, 1978.

DAWOOD, T.; WILLIAMS, M.R.I.; FULLERTON, M.J.; MYLES, K.; SCHUIJERS, J.; FUNDER J.W.; SUDHIR, K.; KOMESAROFF, P.A. Glucocorticoid responses to stress in castrate and testosterone-replaced rams. Regul Pept., v. 125, p. 47-53. 2005.

FÁVARO, W.T.; PADOVANI, C.R.; CAGNON, V.H.A. Ultrastructural and proliferative features of the ventral lobe of the prostate in non-obese diabetic mice (NOD) following androgen and estrogen replacement associated to insulin therapy. Tissue Cell, v. 2, p. 119-132, 2008.

FUSHIMI, H.; HORIE, H.; IMOUE, T.; KAMEYAMA, M.; KANAO, K.; ISHIHARA, S.; TSUJIMURA, T.; NUNOTANI, H.; MINAMI, T.; OKAZAKI, Y.; TOCHINO, Y. Low testosterone levels in diabetic men and animals: a possible role in testicular impotence. Diabetes Res Clin Prac., v. 6, p. 297-301. 1989.

GOODMAN-GRUEN, D.; BARRET-CONNOR, E. Sex diffrence in the association of endogenous sex hormone levels and glucose tolerance status in older men and women. Diabetes Care., v. 23, p. 912-918. 2000.

HO, S.M. Prostatic androgen receptor and plasma testosterone levels in strptozotocin-induced diabetic rats. J Steroid Biochem Molec Biol. v. 38, p. 67-72, 1991.

HUANG, H.; TINDALL, D.J. The role of the androgen receptor in prostate cancer. Crit Rev Eukaryot Gene Expr., v. 12, p. 193-207. 2002.

LAMERS, M.L.; GIMENES, F.A.; NOGUEIRA, F.N.; NICOLAU, J.; GAMA, P.; SANTOS, M.F. Chronic hyperglycaemia increases TGFbeta2 signaling and the expression of extracellular matrix proteins in the rat parotid gland. Matrix Biol., v. 26. p . 572-82, 2007.

LIN, Y.; BERG, A.H.; IYENGAR, P.; LAM, T.K.T.; GIACCA, A.; COMBS, T.P.; RAJALA, M.W.; DU, X.D.; LI, W.; HAWKINS, M.; BARZILAI, N.; RHODES, C.J.; FANTUS, L.G.; BROWNLEE, M.; SCHERER, P.E. The Hyperglycemia-induced Inflammatory Response in Adipocytes. The role of reactive oxygen species. J Biol Chem., v.280, p. 4617–4626. 2005.

MARKER, P.C.; DONJACOUR, A.A.; DAHIYA, R.; CUNHA, G.R. Hormonal cellular and molecular control of cprostatic development. Develop. Biol., v. 253, p.165-174. 2003.

ORDÓÑEZ, P.; MORENO, M.; ALONSO, A.; FERNÁNDEZ, R.; DÍAZ, F.; GONZÁLEZ, C. Insulin sensitivity in streptozotocin-induced diabetic rats treated with different doses of 17beta-oestradiol or progesterone. Exp Physiol., v. 92, n. 1, p. 241-249, 2007.

QUIGLEY, C.A.; DEBELLIS, A.; MARSCHKE, K.B., EL-AWADY, M.K., WILSON, E.M.; FRENCH, F.S. Andorgen receptor defects: historical, clinical and molecular perspectives. Endocr Rev., v. 16, p. 271-321. 1995.

RAMAMANI, A.; ARULDHAS, M.M.; GOVINDARAJULU. P. Differential response of rat skeletal muscle glycogen metabolism to testosterone and estradiol. Can J Physiol Pharmacol., v. 77, p. 300-304, 1999.

REIS, J.S.; VELOSO, C.A.; MATTOS, R.T.; PURISH, S.; NOGUEIRA-MACHADO. Estresse oxidativo: revisão da sinalização metabólica no diabetes tipo 1. Arq Bras Endocrinol Metab., v. 52, n.7,p. 1096-1105, 2008.

RIBEIRO, D.L.; CALDEIRA, E.J.; CÂNDIDO, E.M.; MANZATO, A.J., TABOGA SR, CAGNON VH. Prostatic stromal microenvironment and experimental diabetes. Eur J Histochem., v. 50, p. 51-60, 2006.

RIBEIRO, D.L.; MARQUES, S.F.G.; ALBERTI, A.; SPADELLA, C.T.; MANZATO, A.J.; TABOGA, S.R.; DIZEYI, N.; ABRAHAMSSON, P.A.; GOES, R.M. Malignant lesions in the ventral prostate of alloxan-induced diabetic rats. Int J Exp Patol. v. 89, p. 276-283, 2008b.

RIBEIRO, D.L.; RAFACHO, A.; BOSQUEIRO, J.R.; TABOGA, S.R.; GÓES, R.M. Cellular Changes in Prostatic Stroma of Glucocorticod-treated rats. Cell Tissue Res., v. 332, p. 499-508, 2008a.

RIBEIRO, D.L.; TABOGA, S.R.; GÓES, R.M. Diabetes induces stromal remodelling and increase in chondroitin sulphate proteoglycans of the rat ventral prostate. Int J Exp Pathol. v. 90, n. 4, p. 400-411, 2009.

RISBRIDGER, G.P.; BIANCO, J.J.; ELLEM, S.J.; MC PHERSON, S.J. Oestrogens and prostate cancer. Endocr Relat Cancer., v.10, p.187-191, 2003.

SAITO, M.; NISHI, K.; FOSTER JUNIOR, H.E.; WEISS, R.M.; LATIFPOUR, J. Effect of experimental diabetes on rat prostate endothelin receptors. Eur. J. Pharm., v 310, p. 197-200, 1996.

SAUCIER, J.; DUBE, J.Y.; TREMBLAY, R.R. specific insulin binding sites in the rat testis: characterization and variation. Endocrinology., v. 109, p. 2220-2225, 1981.

SMITH, U.; GALE, E.A. Cancer and diabetes: are we ready for prime time? Diabetologia.,v. 53, n. 8, p. 1541-1544, 2010.

SOUDAMANI, S.; YUVARAJ, S.; MALINI, T.; BALASUBRAMANIAN. K. Experimental diabetes has adverse effects on the differentiation os ventral prostate during sexual maturation os rats. Anat Rec A Discov Mol Cell Evol Biol., v. 287, n. 2, p. 1281-1289. 2005.

SUDHA, S.; SANKAR, B.R.; VALLI, G.; GOVINDARAJULU, P.; BALASUBRAMANIAN, K. Streptozotocin-diabetes impairs prolactin binding to Leydig cells in prepubertal and pubertal rats. Horm Metab Res., v. 31, p. 583-586, 1999.

SUTHAGAR, E.; SOUDAMANI, S.; YUVARAJ, S.; ISMAIL KHAN, A.; ARULDHAS, M.M.; BALASUBRAMANIAN, K. Effects of streptozotocin (STZ)-induced diabetes and insulin replacement on rat ventral prostate. Biomed Pharmacother,Doi: 10.1016/j.biopha.2008.01.2002, 2008.

YONO, M., MANE, S.M., LIN, A., WEISS, R.M., LATIFPOUR J. Differential effects of diabetes induced by streptozotocin and that develops spontaneously on prostate growth in Bio Breeding (BB) rats. Life Sci., v. 83, n. 5-6, 192-197, 2008.

WEEKS, I.; WOODHEAD, J.S. Chemiluminescence immunoassays, J Clin Immunoassays., v.7, p. 82–89, 1984.

WEIHUA, Z.; MAKELA, S.; ANDERSSON, L.C.; SALMI, S.; SAJI, S.; WEBSTER, J.I.; JENSEN, E.V.; NILSSON, S.; WARNER, M.; GUSTAFSSON, J.A. A role for estrogen receptor

beta in the regulation of growth of the ventral prostate. Proc Natl Acad Sci., v. 98, p.6330-6335, 2001.

WILSON, J.D.; GRIFFIN, J.E.; RUSSEL, D.W. Steroid 5αreductase-2 deficiency. Endocr Rev v. 14, p. 577-593, 1993.

Conclusões

As análises dos efeitos em médio termo do diabetes induzido por estreptozotocina sobre a próstata levou às seguintes conclusões:

1) O aumento nos níveis apoptóticos nos animais diabéticos coincidiu com maior peroxidação lipídica e expressão de CML, o que indica aumento do estresse oxidativo e dos AGE, respectivamente. Isso sugere que junto à redução androgênica, o estresse oxidativo também favorece o desequilíbrio nas taxas de proliferação e morte celular da próstata nesta doença;
2) Essa desordem metabólica causou drástica redução nos níveis androgênicos, na proliferação celular e no número de células andrógeno-responsivas. Esses dados corroboram estudos prévios ao indicar que a involução prostática no diabetes experimental ocorre devido ao aumento de apoptose e redução da proliferação celular; além disso, nossos achados mostram que o efeito negativo nas atividades proliferativas do epitélio acinar prostático observados em curto termo após o diabetes persiste em médio termo;
3) As alterações morfológicas observadas na próstata são intermediárias àquelas descritas para períodos curtos e longos de diabetes, com as células exibindo forma cúbica, um fenótipo de transição entre o normal e o altamente atrófico detectado depois de 3 meses da doença;
4) Apesar dos altos níveis glicêmicos constatados, não houve aumento dos sinais inflamatórios e aparentemente não houve aumento da incidência de PIA (atrofia inflamatória proliferativa) e lesões neoplásticas, sugerindo que esse modelo necessita de um tempo maior para o estabelecimento de lesões malignas e pré-malignas;
5) Um mês de diabetes não é suficiente para alterar os biomarcadores de estresse oxidativo no sangue dos ratos. Porém, um aumento na atividade de GST e CAT foi observado na próstata, o que representa uma tentativa da glândula em reverter esta fase inicial de distúrbio no AOS;

6) A família de GST que é considerada um marcador negativo para lesões malignas na próstata, sendo também um bom indicador da defesa antioxidante inicial da glândula;
7) O tratamento com vitamina C não interfere com a atividade das enzimas antioxidantes e nos níveis de peroxidação lipídica na próstata e no sangue; porém restaurou as taxas apoptóticas, marcação de CML nos grupos diabéticos e a atividade de GST. Entretanto, a vitamina C não restaura os níveis proliferativos bem como os parâmetros biométricos e fisiológicos.

As análises dos efeitos em curto termo do diabetes induzido por aloxana sobre a próstata levou às seguintes conclusões:

1) Verificou-se uma correlação inversa entre o peso prostático, a razão testosterona/estradiol (T/E) e os níveis glicêmicos, nesse modelo de diabetes experimental;
2) Embora as mudanças teciduais na próstata ventral de ratos com diabetes induzido por aloxana em curto prazo não tenham sido tão drásticas quanto às observadas em diabetes crônico, estas foram intensificadas com o aumento da glicemia sendo parcialmente revertidas pelo controle com insulina. Esse trabalho mostrou também que o estado glicêmico modula as mudanças morfológicas na próstata mesmo em animais diabéticos tratados com insulina;
3) A análise dos ratos diabéticos, com ou sem reposição de insulina, em diferentes faixas de glicemia mostrou que as mudanças nos níveis de testosterona e estrógenos foram também proporcionais ao aumento da glicemia;
4) O forte desequilíbrio entre os níveis de testosterona e estradiol observados nesta investigação em curto prazo pode ser um fator que favorece a incidência das lesões malignas nesta glândula em diabetes crônico.

Referências Bibliográficas

ABIKO, T.A.; ABIKO, A.C.; CLERMONT, D. Characterization of retinal leukostasis an hemodynamics in insulin resistance and diabetes: role of oxidants and protein kinase-C activation. Diabetes, v. 52, p. 829–837, 2003.

ALMEIDA, E. A.; BAINY, A.C.D.; MEDEIROS, M.H.G.; DI MASCIO, P. Protective effect of phospholipid hydroperoxide glutathione peroxidase (PHGPx) against lipid peroxidation in mussels Perna perna exposed to different metals. Mar Pollut Bull., v. 49, n. 5-6, p. 386–92, 2004.

AHMED, N.; BAEI-JADIDI, R.; HOWELL, S.K.; BEISSWENGER, P.J.; THORNALLEY, P.J. Degradation products of proteins damaged by glycation, oxidation and nitration in clinical type 1 diabetes. Diabetologia, v. 48, n. 8, p. 1590-1603, 2005.

ANDERSON, L.C.; SULEIMAN, A.H.; GARRET, J.R. Morphological effects of diabetes on the glandular ducts and acini of the rat submandibular gland. Microsc Res Tech., v. 27, p. 61-70,1994.

ANTONIOLI, E. Modulação hormonal do comportamento das células musculares lisas prostáticas *in vitro* e *in vivo*. 77f. Dissertação (Mestrado)- Instituto de Biologia- Universidade Estadual de Campinas, 2003.

ARCOLINO, F.O.; RIBEIRO, D.L.; GOBBO, M.G.; TABOGA, S.R.; GÓES, R.M. Aloxan-induced diabetes increases apoptosis and frequency of p63 positive cells in prostate acinar epithelium. Int J. Exp Pathol., v. 91, p. 144-154, 2010.

ARMAGAN, A.; UZ, E.; YILMAZ, H.R.; SOYUPEK, S.; OKSAY, T.; OZCELIK, N. Effects of melatonin on lipid peroxidation and antioxidant enzymes in streptozotocin-induced diabetic rat testis. Asian J Androl., v. 8, n. 5, p. 595–600, 2006.

AUMULLER G. Morphologic and endocrine aspects of prostatic function. Prostate., v. 4, p. 195-214, 1995.

AVEDANO, G.F.; AGARWAL, R.K.; BASHEY, R.I.; LYONS, M.M.; SONI, B.J.; JIOTHRMAYI, G.N.; REGAN, T.J. Effects of glucose intolerancae on myocardial function and collgen-linked glycation. Diabetes, v. 48, p. 1443-1447, 1999.

BAGCHI, D.; VUCHETICH, P.J.; BAGCHI, M.; HASSOUN, E.A.; TRAN, M.X.; TANG, L.; STOHS, S.J. Induction of oxidative estress by chronic administration of sodium dichromate [cadmium H] to rats. Free Radical Biol. Med., v, 22, p. 471-478, 1997.

BANERJEE, P.P.; BANERJEE, S.; LAI, J.M.; STRANDBERG, J.D.; ZIRKIN, B.R.; BROWN, T.R. Age dependent and lobe-specific spontaneous hyperplasia in the Brown Norway rat prostate. Biol Reprod., v. 59, p.1163-1170, 1998.

BAYNES, J.W.; WATKINS, N.G.; FISCHER, C.I.; HULL, C.J.; PATRICK, J.S.; AHMED, M.U.; DUNN, J.A.; THORPE, S.R. The Amadori product on protein: structure and reactions. Prg. Clin. Biol. Res., v. 304, p. 43-67, 1989.

BURKE, J.P.; JACOBSON, D.J.; McGREE, M.; ROBERTS, R.A.; GIRMAN, C.; LIEBER, M.M.; JACOBSERN, S.J. Diabetes and benign prostatic hyperpasia progression in Olmsted county, Minnesota. Adult Urol., v. 67, p. 22-25, 2006.

CAMPBELL, M.K. Bioquímica. Artmed Editora, Porto Alegre, 753, p., 2000.

CAGNON, V.H.A.; CAMARGO, A.M.; ROSA, R.M.; FABIANI, C.R.; PADOVANI, C.R.; MARTINEZ, F.E. Ultraestructure study of the ventral lobe of the prostate of mice with streptozotocin induced diabetes (C57bl/6j).Tissue Cell., v. 32, p. 275-283, 2000.

CALAME, S.S.; LOSTROH, A.J. Effect of insulin and lack of effect of testosterone on the protein of ventral prostate from castrated mice maintained as organ cultures. Endocrinology., v. 76, p. 451-454, 1964.

CAMERON, N.E.; COTTER, M.A. Effects of antioxidants on nerve and vascular dysfunction in experimental diabetes. Diabetes Res. Clin. Pract,, v. 45, p. 137–146, 1999.

CARVALHO, C.A.F.; CAMARGO, A.M.; CAGNON, V.H.A.; PADOVANI, C.R. Effects of experimental diabetes on the structure and ultrastructure of the coagulating gland of C57BL/6j and NOD mice. Anat Rec., v. 270, p. 129-136, 2003.

CARVALHO, H.F.; LINE, S.R.P. Basement membrane associated changes in the rat ventral prostate following castration. Cell Biol Int., v. 20, p. 809-819, 1996.

CARVALHO, H.F.; TABOGA, S.R.; VILAMAIOR, P.S.L. Colagen type VI is a component of the extracellular matrix mocrofibril network of the prostatic stroma. Tissue Cell., v. 29, n. 2, p. 163-170, 1997b.

CARVALHO, H.F.; VILAMAIOR, P.S.L.; TABOGA, S.R. Elastic system of the rat ventral prostate and its modifications following orchiectomy. Prostate., v. 32, n. 1, p. 27-34, 1997a.

CHANDRASHEKAR, V.; STEGER, R.W.; BARTKE, A.; FADDEN, C.T.; KIENAST, S.G. Influence of diabetes on the gonadotropin response to the negative feedback effect of testosterone and hypothalamic neurotransmitter turnover in adult male rats. Neuroendocrilogy., v. 54, n. 1, p. 30-35, 1991.

CHEATHAM, B.; KAHN, C.R. Insulin action and the insulin signaling network. Endocr. Rev., v. 16, p.117-142, 1995.

CHEN, B.H.; JIANG, DE-YONG, J.; TANG, L. Advanced glycation end-products induce apoptosis involving the signaling pathways of oxidative stress in bovine retinal pericytes. Life Sci., v. 79, n. 2, p.1040–1048, 2006.

CHEN, X.; THAKKAR, H.; TYAN, F.; GIM, S.; ROBINSON, H.; LEE, C.; PANDEY S.K.; NWOKORIE, C.; ONWUDIWE, N.; SRIVASTAVA, R.K. Constitutively active Akt is an important regulator of TRAIL sensitivity in prostate cancer. Oncogene., v. 20, n. 42, p. 6073-6083, 2001.

COOKE, P.S.; YOUNG, P.; CUNHA, G.R. Androgen receptor expression in developing male reproductive organs. Endocrinology. v. 128, p. 2867-2873, 1991.

COSTA, M.M.; GOES, R.M.; TABOGA, S.R.; BOSQUEIRO, J.R. Inhibition of insulin signaling pathway and decrease of prostatic epithelial cell proliferation in glucorticoid-treated rats. Am J Physiol Endocrinol Metabol., submetido.

CUNHA, G.R.; ALARID, E.T.; TURNER, T.; DONCAJOUR, A.A., BOUTIN, E.L.; FOSTER, B.A. Normal and abnormal development of the male urogenital tract: role of androgens, mesenchymal-epithelial interactions and growth factors. J Androl., v. 13, p. 465-475, 1992.

CUNHA, R.G., COOKE, P.S., KURITA, T. Role of estromal-epithelial interactions in hormonal responses. Arch Histol Cyto., v. 67, n. 5, p. 417-434, 2004.

CUNHA, G.R.; DONJACOUR, A.A.; COOKE, P.S.; MEE, S., BIGSBY, R.M.; HIGGINS, S.J.; SUGIMURA, Y. The endocrinology and developmental biology of the prostate. Endocr Rev., v. 8, p. 338–362. 1987.

CUNHA, G.R.; HAYWARD, S.W.; DAHIYA, R.; FOSTER, B.A. Smooth muscle-epithelial interactions in normal and neoplastic prostatic development. Acta Anat, v. 155, p. 63-72, 1996.

CUNHA, G.R.; LUNG, B. The possible influences of temporal factors in androgenic responsiveness of urogenital tissue recombinants form wild-type and androgen-insensitive (Tmf) mice. J Exp Zool., v. 205, p. 181-194. 1978.

CROWE, M.J.; FORSLING, M.L.; ROLLS, B.J.; PHILLIPS, P.A.; LEDINGHAM, J.G.; SMITH, R.F. Altered water excretion in healthy elderly men. Age Ageing, v. 16, n. 5, p. 285-293, 1987.

DE MARZO, A.M.; NELSON, W.G.; MEEKER, A.K.; COFFEY, D.S. Stem cell features of benign and malignant prostate epithelial cells. J Urol., v. 160, p. 2381–2392, 1998.

DENIS, U.; LECOMTE, M.; PAGET, C.; RUGGIERO, D.; WIEMSPERGER, N.; LAGARDE, M. Advanced glycation end-products induce apoptosis of bovine retinal pericytes in culture: involvement of diacylglycerol/ceramide production and oxidative stress induction. Free Radicl Biol & Medic., v. 33, p. 236–247, 2002.

DEYL, Z.; MIKSÍK, I. Post-translational non-enzimatic modification of proteins. I. Chromatography of marker adducts with special emphasis to glycation reactions. J. Chromatogr B Biomed Sci Appl., v. 699, n. 1-2, p. 287-309, 1997.

DONCAJOUR, A.A.; CUNHA G.R. Assessment of prostatic protein secretion in tissue recombinants made of urogenital sinus mesenchyme and urothelium from normal or androgen-insensitive mice. Endocrinology., v. 131, p. 2342-2350, 1993.

DOWNWARD, J. Mechanisms and consequences of activation of protein kinase B/Akt. Curr. Opin. Cell Biol., v. 10, p. 262-267, 1998.

DU, X.; STOTCKLAUSER-FARBER, K.; ROSEN, P. Generation of reactive oxygen intermediates, activation of NF-kappaB, and induction of apoptosis in human endothelial cells by glucose: role of nitric oxide synthase? Free Radic Biol Med., v. 27, p. 752–763, 1998.

FÁVARO, W.T.; PADOVANI, C.R.; CAGNON, V.H.A. Ultrastructural and proliferative features of the ventral lobe of the prostate in non-obese diabetic mice (NOD) following androgen and estrogen replacement associated to insulin therapy. Tissue Cell, v. 2, p. 119-132, 2008.

FERNANDES, G.S.; FERNANDEZ, C.D.B.; ASSUMPÇÃO, T.A., TEIXEIRA, C.T.; PEDROSA, F.P.C.; CAMPOS, K.E.; DAMASCENO, D.C.; KEMPINAS, W.G. Reduce oxidative stress in epididymal morphological alteration by antioxidants in hyperglycemic adult male rats: efficiency of vitamin C. Anais do II Workshop on Male Reproductive Biology, 14, P22. 2009.

FLAKOLL, P.J.; CARLSON, M.G.; CHERRINGTON, A.D. Physiological action of insulin. In: LeRoith D, Taylor SI, Olefsky JM, editors. Diabetes mellitus: a fundamental and clinical text. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; p. 148-160, 2000.

GAGO-DOMINGUEZ, M.; CASTELAO, J.E.; PIKE, M.D. Role of Lipid Peroxidation in the Epidemiology and Prevention of Breast Cancer. Can Epid Biom & Prev., v. l, p. 2829–2839, 2005.

GARCIA-FLÓREZ, M.; CARVALHO, H.F. Célula epitelial prostática. In: Células – uma abordagem multidisciplinar. Manole, Barueri. p. 335-345, 2005.

GOBBO, M.G.; TABOGA, S.R.; RIBEIRO, D.L.; GÓES, R.M. Short-term stromal alterations in the rat ventral prostate following alloxan-induced diabetes and influence of insulin replacement. Cell and Tissue Res, Submetido.

GOES, R.M.; ZANETONI, C.; TOMIOSSO, T.C.; RIBEIRO, D.L.; TABOGA, S.R. Surgical and chemical castration induce differential histological response in prostate lobes of Mongolian gerbil. Micron. v. 1111, p. 1-6, 2006.

GONZÁLEZ-PEREZ, A.; GARCÍA RODRÍGUEZ, L.A. Prostate cancer risk among men with diabetes mellitus. Cancer Causes Control., v. 16, p. 1055-1058, 2005.

GUPTA, R.; DHAKAL, B.; THAKUR, A.; AHNN, J. Vitamin C and vitamin E protect the rat testes from cadmium-induced reactive oxygen species. Mol Cells., v.17, p. 132-139, 2004.

GUPTA, R.; SHARMA, R.; CHAUDHARY, R.; YADAV, R.; KHAN, T. Effect of textile waste water on the spermatogenesis of male albino rats. J Appl Toxicol., v. 23, p. 171-175, 2003.

HA, T.S.; CHANG-JU, S.; LEE, J.H. Effects of advanced glycosylation endproducts on perlecan core protein of glomerular epithelim. Pediatr Nephorol., v.19, p. 1219-1224, 2004.

HAYASHI, N.; YOSHIKI, S.; KAWAMURA, J.; DONCAJOUR, A.A.; CUNHA, G.R. Morphological and functional heterogeneitu in the rat prostate gland. Biol Reprod., v. 45, p. 308-321, 1991.

HAYWARD, S.W.; BASKIN, L.S.; HAUGHNEY, P.C.; FOSTER, B.A.; CUNHA, A.R.; DAHIYA, R.; PRINS G.S.; GUNHA, G.R. Stromal development in the rat ventral prostate, anterior prostate and seminal vesicle. Acta Anat., v. 155, p. 94-103, 1996.

HUDSON, D.L.; GUY, A.T.; FRY, P.; O'HARE, M.J.; WATT, F.M., MASTERS, J.R. Epithelial cell differentiation pathways in the human prostate: identification of intermediate phenotypes by keratin expression. Histochem Cytochem., v. 49, n. 2, p. 271-278, 2001.

IKEDA, K.; HIGASHI, T.; SANO, H.; JINNOUCHI, Y.; YOSHIDA, M.; ARAKI, T.; UEDA, S.; HORIUCHI, S. Carboxymethyllysine protein adduct is a major immunological epitope in proteins modified with AGEs of the Maillard reaction. Biochemistry, v. 35, p. 8075–8083, 1996.

INOKI, K.; LI, Y.; XU, T.; GUAN, K.L. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. Genes Dev., v. 17, p. 1829-1834, 2003.

ISAACS, J.T.; COFFEY, D.S. Etiology and disease process of benign prostatic hyperplasia. Prostate Suppl; 2:33-50, 1989.

JENA, A.V.G.; RAMARÃO. P. Insulin-resistence and benign prostatic hyperplasia: The connection. Eur. J. Pharmacol., v. 651, p. 75-81, 2010.

JORGE, A.G.; MÓDULO, C.M.; DIAS, A.C.; BRAZ, A.M.; FILHO, R.B.; JORDÃO, A.A. Jr; de PAULA, J.S.; ROCHA, E.M. Aspirin prevents diabetic oxidative changes in rat lacrimal gland structure and function. Endocr., v. 35, n. 2, p. 189-197, 2009.

KAHN, C.R. The molecular mechanism of insulin action. Ann Rev. Med., v. 36, p. 429-451, 1985.

KERR, J.F.R.; SEARLE J. Deletion of cells by apoptosis during castration–induced involution of the rat prostate. Virchows Arch (B)., v. 13, p. 87-102, 1973.

KLIMCZAK, J.; WISNIEWSKA-KNYPL, J.; KOLAKOWSKI, J. Stimulation of lipid peroxidation and heme oxygenase activity with inhibition of cytochrome P-450 monooxygenase in the liver of rats repeatedly exposed to cadmium. Toxicology., v. 32, p. 257-276, 1984.

KOLODNY, R.C.; KAHN, C.B.; GOLDSTEIN, H.H.; BARNETT, D.M. Sexual dysfunction in diabetic men. Diabetes., v. 23, p. 306-309, 1974.

KOYA, D.; KING, G. L. Protein kinase C activation and the development of diabetic complications. Diabetes, v. 47, p. 859– 866. 1998.

KOYA, D.; LEE, I.K.; ISHII, H. Prevention of glomerular dysfunction in diabetic rats by treatment with d-alpha-tocopherol. J. Am. Soc. Nephrol., v. 8, p. 426–435. 1997.

KOTRIKADZE, N., ALIBEGASHVILI, M., ZIBZIBADZE, M., ABASHIDZE, N., CHIGOGIDZE, T., MANAGADZE, L., ARTSIVADZE, K. Activity and content of antioxidant enzymes in prostate tumors. Exp. Onc., v. 30, n. 3, p. 244-247, 2008.

KOYU, A.; GOKCIMEN, A.; OZGUNER, F.; BAYRAM, D.; KOCAK, A Evaluation of the effects of cadmium on rat liver. Mol Cell Biochem., v. 284, p. 81-85, 2006.

KOTRIKADZE, N., ALIBEGASHVILI, M., ZIBZIBADZE, M., ABASHIDZE, N., CHIGOGIDZE, T., MANAGADZE, L., ARTSIVADZE, K. Activity and content of antioxidant enzymes in prostate tumors. Exp. Onc,. v. 30, n. 3, p. 244-247, 2008.

KOYUTURK, M.; YANARDAG, R,; BOLKENT, S.; TUNALI, S. Infuence of combined antioxidant against cadmium induced testicular damage. Environmental Toxicol Pharmacol., v. 21, p. 235-240, 2006.

KUNIYASU, H., OUE, N., WAKIKAWA, A., SHIGEISHI, H., MATSUTANI, N., KURAOKA, K., ITO, R., YOKOZAKI, H., YASUI, W. Expression of receptors for advanced glycation end-products (RAGE) is closely associated with the invasive and metastatic activity of gastric cancer. J Pathol.,v.196, p. 163–170. 2002.

KURITA, T., WANG, Y.Z., DONJACOUR, A.A., ZHAO, C., LYDON, J.P., O´MALLEY, B.W., ISAACS, J.T., DAHIVA, R., CUNHA, G.R. Paracrine regulation of apoptosis by steroid hormones in the male and female reproductive system. Cell Death Differ*., v.* 8, p. 192-200, 2001.

KYPRIANOU, N.; ISAACS, J.T. Activation of programmed cell death in the rate ventral prostate after castration. Endocrinol.; v. 122, p. 552-562, 1988.

LAMERS, M.L.; GIMENES, F.A.; NOGUEIRA, FN.; NICOLAU, J.; GAMA, P.; SANTOS, M.F. Chronic hyperglycaemia increases TGFβ2 signaling and the expression of extracellular matrix proteins in the rat parotid gland. Matrix Biology. v. 26, p. 572–582, 2007.

LAZAROW, A. Spontaneous recovery from alloxan diabetes in rats. Diabetes., v. 1, p. 363-370, 1952.

LENZEN, S. The mechanisms of alloxan- and streptozotocin induced diabetes. Diabetologia v. 51, p. 216–226, 2008.

LIU, A.Y.; TRUE, L.D.; LATRAY, L. Cell-cell interaction in prostate gene regulation and cytodifferentiation. Proc Natl Acad Sci., v. 94, p. 10705-10710, 1997.

LOSTROH, A.J. Effect of testosterone and insulin *in vitro* on maintenance and repair of the secretory epithelium of the mouse prostate. Notes and Comments, v. 88, p. 501-503, 1970.

MANCA, D.; RICHARD, A.C.; TROTTIER, B.; CHEVALIER, G. Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. Toxicology., v. 67, p. 303-323, 1991.

MALLIDIS, C.; AGBAJE, I.M.; ROGERS,D.A.; GLENN, J.V.; PRINGLE, R.A.; ATKINSON, B.; STEGER, K.; STITT, A.W.; MCCLURE, N. Advanced glycation end products accumulate in thereproductive tract of men with diabetes. Int J Androl., v. 32, p. 295-305, 2007.

MARKER, P.C.; DONJACOUR, A.A.; DAHIYA, R.; CUNHA, G.R. Hormonal cellular and molecular control of prostatic development. Develop Biol., v. 253, p. 165-174, 2003.

MARKS, L.S.; MOSTAGHEL, E.A.; NELSON, P.S. Prostate tissue androgens: history and current clinical relevance. Urology., v.72, n. 2, p. 247-254, 2008.

McNEAL, J.E. Prostate. In: Histology for Pathologists. Lippincott-Raven Publishers, Philadelfia. Cap. 42. 1997.

MCCANCE, D.R.; HANSON, R.L.; PETTITT, D.J.; BENETT, P.H., HADDEN, D.R.; KNOWLER, WC. Diagnosing diabetes mellitus – do we need new criteria? Diabetologia, v. 40, p. 247-255, 1997.

MONNIER, VM; SELL, DR; NAGARAI, RH; MIYATA, S; ODETTI, P; IBRAHIM, SA. Maillard reaction-mediated molecular damage to extracellular matrix and other tissue proteins in diabetes, aging, and uremia. Diabetes., v. 2, p. 36-42, 1992.

MONTES, G.S. Distribution of oxytalan, elaunin and elastic fibers in tissues. Cienc. Cult., v. 44, n. 4, p. 224-233, 1992.

NARUSE, K.; NAKAMURA, J.; HAMADA, Y.; NAKAYAMA, M.; CHAYA, S.; KOMORI, T.; KATO, K.; KASUYA, Y.; MIWA, K.; HOTTA, N. Aldose reductase inhibition prevents glucose-induced apoptosis in cultured bovine retinal microvascular pericytes. Exp Eye Res., v. 71, p. 309–315, 2000.

OBERLEY, T.D.; ZHONG W.; SZWEDA, L.I.; OBERLEY, L.W. Localization of Antioxidant Enzymes and Oxidative Damage Products in Normal and Malignant Prostate Epithelium. The Prostate. v. 44, p. 144–155, 2000.

OGA, S. Fundamentos de Toxicologia. Atheneu Editora, São Paulo, 774 p., 2003.

O'DONNELL, V. B.; SPYCHER, S.; AZZI, A. Involvement of oxidants and oxidant-generating enzyme(s) in tumour-necrosis-factor-alpha- mediated apoptosis: role for lipoxygenase pathway but not mitochondrial respiratory chain. Biochem. J., v.310, p. 133–141; 1995.

OLIVERIA, S.M.; LEITE VILAMAIOR, P.S.; CORRADI, L.S.; GÓES, R.M.; TABOGA, S.R. Cellular and extracellular behavior in the gerbil (Meriones unguiculatus) ventral prostate following different types of castration and the consequences of testosterone replacement. Cell Biol Int., v. 31, n. 3, p. 235-45, 2007.

ORDÓÑEZ, P.; MORENO, M.; ALONSO, A.; FERNÁNDEZ, R.; DÍAZ, F.; GONZÁLEZ, C. Insulin sensitivity in streptozotocin-induced diabetic rats treated with different doses of 17beta-oestradiol or progesterone. Exp Physiol., v. 92, n. 1, p. 241-249, 2007.

ÖZTÜRK, Y.; ALTAN, V.M.; YILDIZOGLU-ARI, N. Effects of experimental diabetes and insulin on smooth muscle functions. Pharmacol Rev., v. 48, p. 69-104, 1996.

POLYCHRONAKOS, C.; JANTHLY, U.; LEHOUX, J.G.; KOUTSILLIERS, M. Mitogenic effects of insulin and insulin-like growth factors on PA-III rat prostate adenocarninoma cells: characterization of the receptors involved. Prostate., v. 19, p. 313-321, 1991.

RAHIMI, R.; NIKFAR, S.; LARIJANI, B.; ABDOLLAHI, M. A review on the role of antioxidants in the management of diabetes and its complications. Biomed Pharmacother., v. 59, n. 7, p. 365-373, 2005.

RASLOVA, K. An update on the treatment of type 1 and type 2 diabetes mellitus: focus on insulin determir, a long acting human insulin analog. Vasc Health Risk Manag., v. 6, p. 399–410, 2010.

REIS, J.S.; VELOSO, C.A.; MATTOS, R.T.; PURISH, S.; NOGUEIRA-MACHADO. Estresse oxidativo: revisão da sinalização metabólica no diabetes tipo 1. Arq Bras Endocrinol Metab., v. 52, n.7,p. 1096-1105, 2008.

RIBEIRO, D.L.; CANDIDO, E.M.; CALDEIRA, E.; MANZATO, A.J.; TABOGA, S.R.; CAGNON, V.H.A. Prostatic stromal microenvironment and experimental diabetes. Eur J Histochem., v. 5, p. 51-60, 2006.

RIBEIRO, D.L.; MARQUES, S.F.; ALBERTI, S.; SPADELLA, C.T.; MANZATO, A.J.; TABOGA, S.R.; DIZEYI, N.; ABRAHAMSSON, P.A.; GÓES, R.M. Malignant lesions in the ventral prostate of alloxan-induced diabetic rats. Int J Exp Pathol., v. 89, n. 4, p. 276-283, 2008 .

RIBEIRO, D.L.; TABOGA, S.R.;GÓES, R.M. Diabetes induces stromal remodelling and increase in chondroitin sulfate proteoglycans of the rat ventral prostate. Int J Exp Pathol., v. 90, n. 4, p. 400-411, 2009.

RISBRIDGER, G.P., BIANCO, J.J., ELLEM, S.J., MC PHERSON, S.J. Oestrogens and prostate cancer. Endocr Relat Cancer., v. 10, p. 187-191, 2003.

ROCHEL, S.S.; BRUNI-CARDOSO, A., TABOGA, S.R., VILAMAIOR, P.S., GÓES R.M. Lobe identity in the Mongolian gerbil prostatic complex: a new rodent model for prostate study. Anat Rec (Hoboken)., v. 10, p. 1233-1247. 2007.

SABATINI, D.M. mTOR and cancer: insights into a complex relationship. Nat. Rev. Cancer., v. 6, p. 729-734, 2006.

SANAI, T.; SOBKA, T.; JOHNSON, T.; ESSAWY, M.E.I.; MUCHANETA, K.; GHARBIA, O.B.; OLDROYD, S.; NAHAS, A.M.E. Expression of citoeskeletal proteins uring the course of experimental diabetic nephropaty. Diabetologia., v. 14, p. 91-1000, 2000.

SARAFINOVSKA, Z.A.; EKEN, A.; MATEVSKA, N.; ERDEM, O.; SAYAL, A.; SAVASER, A. et al. Increased oxidative/nitrosative stress and decreased antioxidant enzyme activities in prostate cancer. Clin Biochem., v. 42, p.1228–1235, 2009.

SAXENA, A.K.; SAXENA, P.; WU, X.; OBRENOVICH, M.; WEISS, M.F.; MONNIER, V. M. Protein aging by carboxymethylation of lysines generates sites for divalent metal and redox active copper binding: relevance to diseases of glycoxidative stress. Biochem. Biophys. Res. Commun., v. 260, p. 332–338; 1999.

SAUCIER, J.; DUBE, J.Y.; TREMBLAY, R.R. Specific insulin binding sites in the rat prostate. Characterization and variation. Endocrinology., v. 109, p. 2220-2225, 1981.

SCARANO, W.R.; VILAMAIOR, P.S.; TABOGA, S.R. Tissue evidence of the testosterone role on the abnormal growth and aging effects reversion in the gerbil (Meriones unguiculatus) prostate. Anat Rec., v. 288, n. 11, p. 1190-200, 2006.

SCHALKEN J.A.; VAN LEENDERS G. Cellular and molecular biology of the prostate: stem cell biology. Urology., v. 62, p. 11-20. 2003.

SCHINNER, S.; SCHERBAUM, W.A.; BORNSTEIN, S.R.; BARTHEL, A. Molecular mechanisms of insulin resistance. Diabet Med., v. 22, p. 674-682, 2005.

SHRILATHA, B.; MURALIDHARA. Occurrence of oxidative impairments, response of antioxidant defences and associated biochemical perturbations in male reproductive milieu in the Streptozotocin-diabetic rat. Int J Androl., v. 30, p. 508–518, 2007a.

SHRILATHA, B.; MURALIDHARA. Early oxidative stress in testis and epididymal sperm in streptozotocin-induced diabetic mice: Its progression and genotoxic consequences. Reprod Toxicol. v. 23, p. 578–587, 2007b.

SPECTOR, A. Oxidative stress and disease. J Ocul Pharmacol Ther., v. 16, p. 193–201, 2000.

SREEPRIYA M.; BALI, G. Effects of administration of Embelin and Curcumin on lipid peroxidation, hepatic gluthathione antioxidant defense and hematopoietic system during N-nitrosodiethylamine/Phenibarbital-induced hepatocarcinogenesis in Wistar rats. Mol Cell Biochem., v. 284, p. 49-55, 2006.

SINGH, R., BARDEN. A., MORI, T., BEILIN, L. Advanced glycation endproducts: A review. Diabetologia, v. 44, p. 129–146, 2001.

STAAK, A.; KASSIS, A.P.; OLSHEN, A.; WANG, Y.; WU, D.; CARROLL, P.R.; GROSSFELD, G.D.; CUNHA, G.R; HAYWARD, S.W. Quantitation of Apoptotic Activity Following Castration in Human ProstaticTissue *In Vivo*. The Prostate., v. 54, p. 212-219, 2003.

STEFAN, S.F. Definition and classification of diabetes including maturity-onset diabetes of the young. Diabetes Mellitus, A fundamental and clinical text. Cap. 27, p. 251-290. 1996.

STEGE, R.W.; RABE, M.B. The effect of diabetes mellitus on endocrine and reproductive function. Proc Soc Exp Biol Med., v. 214, p. 1-11, 1997.

STITT, A.W.; LI, Y.M.; GARDINER, T.A.; BUCALA, R.; ARCHER, D.B.; VLASSARA, H. Advanced glycation end products (AGEs) co-localize with AGEs receptors in the retinal vasculature of diabetic and of AGE-infused rats. Am J Pathol., v. 150, n. 2, p. 523–531, 1997.

STUDER, R.K.; CRAVEN, P.A; DERUBERTIS, F.R. Antioxidant inhibition of protein kinase C-signaled increases in transforming growth factor-beta in mesangial cells. Metabolism., v. 46, p.918–925. 1997.

SUDHA, S.; RAVISANKAR, V.; JULIE, P.M.; ARUNAKARAN, J.; GOVINDARAJULU, P.; BALASUBRAMANIAN, K. Influence of streptozotocin-induced diabetes and insulin

replacement on the pituitary-testicular axis during sexual maturation in rats. Exp. Clin. Endocrinol Diab., v. 108, p. 14-20. 2000.

SUGIMURA, Y.; CUNHA, G.R.; DONJACOUR, A.A. Morphological and histological study of castration-induced degeneration and androgen-induced regeneration in the mouse prostate. Bio. Reprod., v. 34, p. 973-983, 1986.

SUTHAGAR, E.; SOUDAMANI, S.; YUVARAJ, S.; ISMAIL KHAN, A.; ARULDHAS, M.M.; BALASUBRAMANIAN, K. Effects of streptozotocin (STZ)-induced diabetes and insulin replacement on rat ventral prostate. Biomed Pharmacother., v. 63,n. 1, p. 43-50, 2008.

SZKUDELSKI, T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res., v. 5, n. 6, p. 537-546, 2001.

TAGUCHI A., BLOOD, D.C., DEL TORO, G., CANET, A., LEE, D.C., QU, W., INGRAM, M., LU, A., TANAKA, H., HORI, O., OGAWA, S., STERN, D.M., SCHMIDT, A.M. Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. Nature., v.405, p.354–360, 2000.

UENO, M.; CARVALHEIRA, J.B.C.; TAMBASCIA, R.C.; BEZERRA, R.M.N.; AMARAL, M.E.; CARNEIRO, E.M.; FOLLI. F.; FRANCHINI, K.G.; SAAD, M.J.A. Regulation of insulin signalling by hyperinsulinaemia: role of IRS-1/2 serine phosphorylation and the mTOR/p70 S6K pathway. Diabetologia., v. 48, p. 506-518, 2005.

UNTERGASSER, G.; MADERSBACHER, S.; BERGER, P. Benign prostatic hyperplasia: age-related tissue-remodeling. Exp Gerontol., v. 40, p. 121-128, 2005.

VAN DER OOST, R.; BEYER, J.; VERMEULEN, N.P.E. Fish bioaccumulation and biomarkers in environmental risk assessement: a review. Environ Toxicol Pharmacol., v. 13, n. 2, p. 57–149, 2003.

VAN LEENDERS, G.; DIJKMAN, H.; VAN DE KAA, C. H.; RUITER, D.; SCHALKEN, J. Demonstration of Intermediate Cells during Human Prostate Epithelial Differentiation *In Situ* and *In Vitro* Using Triple-Staining Confocal Scanning Microscopy. Lab Invest., v. 80, p.1251–1258, 2001.

WANG, Z.J.; IKEDA, K.; WADA, Y.; FOSTER JR, H.E.; R.M. WEISS; LATIFPOUR, J. Expression and localization of basic fibroblast growth factor in diabetic rat prostate. BJU internacional., v. 85, p. 945-952, 2000.

WAUTIER, M.P.; CHAPPEY, O.; CORDA, S.; STERN, D.M.; SCHMIDT, A.M.; WAUTIER, J.L. Activation of NADPH oxidase by AGE links oxidant stress to altered gene expression via RAGE. American Journal of Physiology. Am J Physiol Endocrinol Metab., v. 280, p. 685–694, 2001.

WHITE, M.F. Insulin signaling in health and disease. Science., v**.** 302, p. 1710-1711, 2003.

WILLIAMSON, J.R.; CHANG, K.; FRANGOS, M.; HASAN, K.S.; IDO, Y.; KAWANURA, T.; NYENGOARD, J.R.; VAN DEN EDDEN, M.; KILO, C.; TILTON, R.G. Hyperglycemic pseudohypoxia and diabetic complications. Diabetes.,v. 42, p. 801–813. 1991.

YAN, S.D.; SCHMIDT, A.M.; ANDERSON, G.M.; ZHANG, J.; BRETT, J.; ZOU, Y.S.; PINSKY, D.; STERN, D. Enhanced cellular oxidant stress by interaction of advanced glycation end products with their receptors/binding proteins. J Biol Chem., v. 269, p. 9889–9897, 1994.

YASSER, H.A.; ABDEL-WAHAB, FINBARR, P.M.; O'HARTE; MARK, H. MOONEY; CHRISTOPHER, R.; BARNETT; PETER, R.; FLATT. Vitamin C Supplementation Decreases Insulin Glycation and Improves Glucose Homeostasis in Obese Hyperglycemic (*ob/ob*) Mice. Metabolism., v. 51, n. 4, p. 514-517, 2002.

YATOH, S.; MASAKAZU, M.; YOKOO, T.; SONE, H.; TOYOSHIMA, H.; SUZUKI, S.; SHIMANO, H.; KAWAKAMI, Y.; OKUDA, Y.; YAMADA, N. Antioxidants and an inhibitor of advanced glycation amelionare death of retinal microvascular cells in diabetic retinopathy. Diabetes Metab Res Rev., v. 22, p. 38-45, 2006.

YENUSH, L., WHITE, M.F. The IRS-signalling system during insulin and cytokine action. Bioessays., v. 19, p. 491-500, 1997.

YONO, M., MANE, S.M., LIN, A., WEISS, R.M., LATIFPOUR J. Differential effects of diabetes induced by streptozotocin and that develops spontaneously on prostate growth in Bio Breeding (BB) rats. Life Sci., v. 83, n. 5-6, 192-197, 2008.

YONO, M.; POURESMAIL, M.; WATARU, T.; FLANAGAN, J.F.; WEISS, R.M.; LATIFPOUR, J. Effect of insulin treatment on tissue size of the genitourinar tract in BB rats with spontaneously developed and streptozotocin-induced diabetes. Naunyn Schmiedebergs Arch Pharmacol., v. 372, p. 251–255, 2005.

ZIAEI-RAD, M.; VAHDANINIA, M.; MONTAZERI, A. Sexual Dysfunctions in Patients with Diabetes – a study from Iran. Reprod. Biol. Endocrinol., v. 8, p. 50, 2010.

ZHANG, J.F.; WANG, X.R.; GUO, H.Y.; WU, J.C., XUE, Y.Q. Effects of water-soluble fractions of diesel oil on the antioxidant defenses of the goldfish, Carassius auratus. Ecotoxicol Environ Saf., v. 58, n. 1, p. 110–6, 2004.

DECLARAÇÃO

Declaro para os devidos fins que o conteúdo de minha **Dissertação de Mestrado** intitulada "**INFLUÊNCIA DA HIPERGLICEMIA E DO ESTRESSE OXIDATIVO NA CINÉTICA DE PROLIFERAÇÃO E MORTE CELULAR NO EPITÉLIO ACINAR DA PRÓSTATA DE RATOS DIABÉTICOS**"

( ) não se enquadra no § 3º do Artigo 1º da Informação CCPG 01/08, referente a bioética e biossegurança.

Tem autorização da(s) seguinte(s) Comissão(ões):

( ) CIBio - Comissão Interna de Biossegurança , projeto nº _____, Instituição _____

(X) CEUA - Comissão de Ética no Uso de Animais , projeto nº **006/2009**, Instituição: **Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de São José do Rio Preto, SP.**

( ) CEP - Comissão de Ética em Pesquisa, protocolo nº _____, Instituição _____

** Caso a Comissão seja externa ao IB/UNICAMP, anexar o comprovante de autorização dada ao trabalho. Se a autorização não tiver sido dada diretamente ao trabalho de tese ou dissertação, deverá ser anexado também um comprovante do vínculo do trabalho do aluno com o que constar no documento de autorização apresentado.*

Marina Guimarães Gobbo

Aluna: Marina Guimarães Gobbo

Orientadora: Profa. Dra. Rejane Máira Góes

Para uso da Comissão ou Comitê pertinente:
( ) Deferido ( ) Indeferido

Carimbo e assinatura

Profa. Dra. ANA MARIA APARECIDA GUARALDO
Presidente da Comissão de Ética no Uso de Animais
CEUA/UNICAMP

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"JÚLIO DE MESQUITA FILHO"
Câmpus de São José do Rio Preto

COMISSÃO DE ÉTICA NA EXPERIMENTAÇÃO ANIMAL

IBILCE

CERTIFICADO

Certificamos que o projeto de pesquisa intitulado "**Influência da hiperglicemia e do estresse oxidativo na cinética de proliferação e morte celular no epitélio acinar da próstata de ratos diabéticos**" (protocolo no. 006/09 CEEA), sob responsabilidade de Rejane Maira Góes, está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Colégio Brasileiro de Experimentação Animal (COBEA), tendo sido aprovado pela Comissão de Ética na Experimentação Animal, em reunião de 24/04/2009.

CERTIFICATE

UNESP / IBILCE Ethical Committee for Animal Research (CEEA) hereby certify that the scientific investigation entitled "**Influência da hiperglicemia e do estresse oxidativo na cinética de proliferação e morte celular no epitélio acinar da próstata de ratos diabéticos**" (protocol n. 006/09 CEEA), on Rejane Maira Góes responsibility, is in accordance with Ethical Principles in Animal Research adopted by Brazilian College of Animal Experimentation (COBEA) and it was approved by the Committee of this Institute, on April 24th, 2009.

São José do Rio Preto, April 24th, 2009.

Profa. Dra. Rejane Maira Góes
Presidente da CEEA

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