DANIEL SILVA SENA BASTOS

EFEITO DA DIETA HIPERGLICÍDICA E DO ANABOLIZANTE UNDECILENATO DE BOLDENONA SOBRE A BIOQUÍMICA DOS LIPÍDIOS, PERFIL DE CITOCINAS, ESTADO OXIDATIVO E MORFOLOGIA CARDÍACA EM CAMUNDONGOS APO-E$^{-/-}$

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Biologia Celular e Estrutural, para obtenção do título de *Magister Scientiae*.

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APROVADO: 19 de fevereiro de 2016.

_____ Solange Mara Bigonha

_____ Bruno Edésio dos Santos Melo

_____
Izabel Regina dos Santos Costa Maldonado
(Orientadora)

Dedico esta dissertação a Deus e a
toda minha família

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SUMÁRIO

RESUMO

BASTOS, Daniel Silva Sena, M.Sc., Universidade Federal de Viçosa, Fevereiro de 2016. **Efeito da dieta hiperglicídica e do anabolizante undecilenato de boldenona sobre a bioquímica dos lipídios, perfil de citocinas, estado oxidativo e morfologia do coração em camundongos Apo-E$^{-/-}$.** Orientadora: Izabel Regina dos Santos Costa Maldonado. Coorientador: Eliziária Cardoso dos Santos.

Neste estudo foram investigados os efeitos do anabolizante undecilenato de boldenona (Bol) e da dieta hiperglicídica (HC) no coração de camundongos Apo-E$^{-/-}$. Quarenta camundongos fêmeas com 10 semanas de idade foram aleatoriamente distribuídos em 6 grupos com 8 animais cada. Controle) C57BL/6; NT) Apo-E$^{-/-}$ + dieta padrão; Bol) Apo-E$^{-/-}$ + dieta padrão + 20 mg/kg BOL; HC Apo-E$^{-/-}$ + HC; Bol 10 e Bol 20) Apo-E$^{-/-}$ + HC + 10 ou 20mg/kg Bol, respectivamente. A dieta HC foi desenvolvida de acordo com protocolo AIN-93G para dietas purificadas. A boldenona foi administrada intraperiteonealmente três vezes por semana, durante oito semanas. Nossos dados indicam que HC e Bol sozinhos ou combinados aumentaram o peso dos animais, as concentrações séricas de LDL, e a atividade da glutationa-s-transferase no coração. Por outro lado, animais tratados com HC e Bol sozinhos apresentaram redução na atividade da catalase. Todos os grupos apresentaram redução das concentrações de malondialdeído. As concentrações séricas da isoenzima creatinoquinase CK-MB foram maiores nos animais tratados com Bol e HC sozinhos, mas não nas combinações. HC aumentou as concentrações séricas de TNF-α, IFN-γ, IL-2, IL-10 e IL-17. Nas combinações o efeito foi variável. A vascularização foi evidente nos tratamentos com Bol e HC sobretudo nas combinações. Enquanto HC sozinha aumentou a presença de infiltrado inflamatório esse efeito foi atenuado em BOL. Juntos esses dados mostram que tanto Bol quanto HC podem independentemente agravar a dislipidemia e induzir o remodelamento do miocárdio. Aparentemente, HC é potencialmente mais perigoso que Bol, induzindo um evidente estado pró-inflamatório sistêmico, que é diretamente relacionado a presença de infiltrado inflamatório. Contudo, Bol e HC podem prejudicar a atividade das enzimas antioxidantes alterando o balanço redox.

ABSTRACT

BASTOS, Daniel Silva Sena, M.Sc., Universidade Federal de Viçosa, February, 2016. **Effect of the high carbohydrate and boldenone undelylenate in biochemistry of lipids and cytokines profile, oxidative status, heart morphology of Apo-E$^{-/-}$ mice.** Adviser: Izabel Regina dos Santos Costa Maldonado. Co-adviser: Eliziária Cardoso dos Santos.

In this study we investigated the effects of high-carbohidrate dietary (HC) intake and boldenone undecylenate (Bol) on lipid and cytokine profiles, redox balance and heart morphology in Apo-E$^{-/-}$ mice. Female mice 10-weeks old, were randomized in 6 group with 8 animals each: Control) C57BL/6 + standard diet; NT) Apo-E$^{-/-}$ + standard diet; Bol) Apo-E$^{-/-}$ + standard dietary + 20 mg/kg Bol; HC) Apo-E$^{-/-}$ + HC; Bol 10 and Bol 20) Apo-E$^{-/-}$ + HSD + 10 or 20mg/kg BOL, respectively. The HSD was developed according the protocol AIN-93G for purified diets. Bol was administered intraperitoneally three times per week, for 8 weeks. Our data indicated that HC and Bol alone or combined increased body weight, LDL and HDL cholesterol serum levels, and cardiac activity of glutathione-s-transferase in Apo-E$^{-/-}$ mice. Conversely, animals treated with HC or Bol alone presented reduced activities of catalase and superoxide dismutase. All groups receiving HC or Bol presented reduced tissue levels of malondialdehyde. The creatine kinase-izoenzyme (CK-MB) levels were high in animals treated with BOL or HSD alone, but not combined. In general, HSD increased TNF-α, IFN-γ, IL-2, IL-10 and IL-17 serum levels. However, this effect was variable in animals receiving HSD plus BOL. Although HSD and BOL have not exercised detectable influence on the morphology of the cardiac parenchyma, tissue vascularization was markedly increased by both treatments, especially when combined and in a dose-dependent way. While HC alone increased myocardial accumulation of inflammatory cells, this effect was attenuated by Bol. Taken together, our findings indicated that both HC and Bol can independently aggravated the dyslipidemia and induce a marked vascular remodeling in cardiac tissue. Apparently, HC is potentially more dangerous that Bol, inducing a marked pro-inflammatory systemic status, which is potentially related to the cardiac inflammatory infiltrate in Apo-E$^{-/-}$ mice. Although HC and Bol can impair the activities of antioxidant enzymes, this effect seem to be harmful to the myocardium redox balance.

1 INTRODUÇÃO GERAL

As doenças cardiovasculares estão entre as principais causas de morbidade e mortalidade no mundo ocidental (Ishigaki *et al*., 2008). A aterosclerose é uma doença cardiovascular mais incidente e, além de ser uma doença de origem múltipla, se caracteriza por alterações no metabolismo lipídico e no processo inflamatório (Moore & Tabas, 2011). Os fatores de risco para desenvolvimento e progressão da doença são: sedentarismo, tabagismo, elevada ingestão de gorduras (gordura trans e saturadas), obesidade, diabetes e também a dislipidemia, síndrome metabólica caracterizados como aumento excessivo de colesterol e/ou triglicérides no sangue (Sociedade Brasileira de Cardiologia, 2007).

A disfunção endotelial é considerada o fator inicial na aterogênese e caracteriza-se por uma resposta inflamatória da parede vascular à injúria Hansson 2005 *et al.*, Kronzon *et al*., 2006; Puri *et al*., 2013). A partir de reações celulares e moleculares como, ataque dos radicais livres e também o aumento das concentrações séricas da lipoproteína de baixa densidade (LDL) e a presença de LDL oxidada (LDLox) na parede arterial são responsáveis pelo comprometimento do endotélio (Kovanen & Pentikainen, 2003; Siqueira *et al*., 2006).

A oxidação da LDL é a ideia, o evento central que desencadearia o início do processo da formação da placa aterosclerótica, sendo o estresse oxidativo evento primordial de início e progressão da doença (Cheng, 2013). Os mecanismos de defesa antioxidante do organismo contra o estresse ocorrem de modo enzimático (catalase, superóxido dismutase, glutationa S-transferase) e não enzimático (Huige *et al*., 2014) a fim de manter o equilíbrio redox no tecido

Um dos fatores de risco bem definidos para o desenvolvimento da aterosclerose é a obesidade causada por dieta, que vem aumento nas sociedades ocidentais (Fleg, 2013). As primeiras associações entre o aumento do colesterol sérico e problemas cardiovasculares surgiram na década de 50 nos Estados Unidos. Consequentemente, as primeiras recomendações para a diminuição da ingestão de gordura, principalmente a saturada, para indivíduos com níveis elevados de colesterol. A partir daí a alimentação saudável passou

a ser definida como uma dieta hipolipídica. Gordura se tornou sinônimo de calorias, e a dieta saudável tornou-se a hipolipídica, porem alimentos *low-fat* (pobres em gorduras) são capazes de promover acúmulo de gordura corporal. Contudo, os produtos *low-fat* disponíveis no mercado podem confundir os consumidores, já que, muitas vezes, contêm grande concentração de açúcar e amido, além de alta densidade energética (Polacow & Lancha Jr., 2007).

Afim de combater e reduzir os riscos cardiovasculares, pesquisadores recomendam a diminuição da gordura saturada na dieta, dieta de alto risco cardiovascular (Polacow & Lancha 2007). Uma das estratégias comumente utilizada para prevenir e combater os riscos cardiovasculares é a dieta hipolipídica, porém rica em carboidratos (dieta hiperglicídica). Diversos pesquisadores sugerem que dietas hiperglicídicas pode aumentar a concentração de triglicérides, hipertrigliceridemia induzida por carboidratos, além de favorecer a formação de LDL ao mesmo tempo que reduz as concentrações de HDL (Graham *et al*., 2010 Sirjani *et al*., 2014).

Para o estudo da aterosclerose, têm ocorrido vários avanços com o uso de camundongos geneticamente modificados, destacando-se o camundongo nocaute para a apolipoproteína E (Apo-$E^{-/-}$) e o nocaute para o receptor de lipoproteína de baixa densidade ($LDLr^{-/-}$) que desenvolvem espontaneamente lesões ateroscleróticas na túnica intima dos vasos de médio e grande calibre e principalmente na aorta proximal. Em ambos os casos, o desenvolvimento da lesão é acelerado quando os animais são conduzidos a uma dieta com alto teor de gordura saturada (Jackson *et al*., 2007).

Os animais Apo-$E^{-/-}$ possuem o colesterol plasmático cerca de cinco vezes mais alto que os normais e desenvolvem, espontaneamente, estrias gordurosas na túnica intima da aorta proximal aos três meses de idade (Zhang *et al*., 1992, Wang *et al* 2015). Camundongos Apo-$E^{-/-}$ com oito semanas, tratados com dieta comercial, já apresentam adesão das células mononucleares e formação esporádica de células espumosas, causando lesões na décima semana de idade. A placa fibrosa aparece com vinte semanas de vida. As lesões podem agravar precocemente pelo consumo de “dietas ocidentais” ou aterogênicas, ricas em gorduras e carboidratos. O surgimento das células espumosas nos animais tratados com dieta aterogênica ocorre, com 8 semanas de vida, com o surgimento da placa fibrosa na décima

quinta semana de vida. (Reddick *et al*., 1994; Nakashima *et al*., 1994; Wang, 2015).

O aumento exacerbado da massa corporal, além de representar um fator predisponente no surgimento de complicações cardiovasculares, também influencia na aparência corporal, gerando a insatisfação das pessoas com sua própria aparência física, o que desencadeia a busca e adoção desenfreada de cuidados com o corpo, envolvendo a musculação, dietas e cirurgias plásticas. Além da aparência corporal como um todo, o culto ao corpo caracteriza-se pela melhoria do desempenho e força física, assim os indivíduos que são adeptos a este culto, principalmente praticantes de musculação, passam a apostar no uso de esteroides anabolizantes (Le Breton, 2003; Iriart, 2009).

O uso indiscriminado de EA tem se mostrado causador de sérias alterações no sistema cardiovascular, como as complicações vasculares, cardiomiopatias, aterosclerose (Melchert & Welber, 1995), hipertensão (Takahashi *et al*., 2004) e aumento do colágeno tecidual (Parssinen *et al*, 2000) caracterizando a hipertrofia cardíaca patológica. Entretanto, seus reais efeitos sobre o tecido cardíaco precisam de maiores esclarecimentos.

Diante do exposto, o presente estudo visa elucidar o efeito do esteroide anabolizante undecilenato de boldenona e da dieta hiperglicídica sobre o perfil lipídico e inflamatório, estado oxidativo, e parâmetros morfológicos do coração de camundongos Apo-$E^{-/-}$.

2 REVISÃO DE LITERATURA

2.1 Camundongos deficientes no gene da apolipoproteína E (Apo-$E^{-/-}$).

Desde a década de 60, as doenças cardiovasculares vêm apresentando aumento progressivo em todo mundo, sendo as dislipidemias consideradas como um dos fatores determinantes para o desenvolvimento dessas enfermidades, principalmente a aterosclerose (Grillo *et al*., 2005). Durante os últimos anos houve um declínio razoável da mortalidade por causas cardiovasculares em países desenvolvidos, enquanto há elevação consideravelmente rápida desta taxa em países em desenvolvimento, como o Brasil (Sociedade Brasileira de Cardiologia, 2007).

As investigações clínicas, os estudos populacionais, bem como experimentos com culturas de células têm fornecido importantes indicadores na

patogênese da aterosclerose. Contudo, devido às grandes dificuldades em estudar os mecanismos patogênicos da aterosclerose em humanos, tornou-se fundamental a presença de um modelo experimental bem caracterizado para esta patologia (Hansson *et al*., 2005).

Os camundongos modificados geneticamente pela deleção de um alelo específico responsável por codificar uma proteína com alto grau de especificidade. O símbolo $^{-/-}$ é utilizado para se referir a animais geneticamente modificados. Os camundongos mais utilizados para o estudo da aterosclerose são nocautes para o gene da apolipoproteína E (Apo-E) ou do receptor para LDL (LDLr), ambos essenciais para o metabolismo lipídico (Daugherty, 2002). A Apo-E é uma glicoproteína rica em arginina com peso molecular de 34 kdaltons, sintetizada principalmente no fígado e cérebro. Por ser um constituinte da superfície de lipoproteínas, principalmente VLDL, HDL e quilimícrons, a Apo-E desempenha um papel funcional na depuração (*clearence*) das lipoproteínas plasmáticas (Davignon *et al*., 1999).

Apo E também pode ser sintetizada por monócitos e macrófagos nos vasos sanguíneos, apresentando efeitos locais na homeostase do colesterol e nas reações inflamatórias que ocorrem nos vasos ateroscleróticos, devido ao seu papel na imunomodulação, que afeta a secreção de várias citocinas. Além disso, a Apo E inibe a agregação plaquetária, exerce efeitos antiproliferativos, contribui para o efluxo de colesterol das células nas lesões ateroscleróticas e possui propriedades antioxidantes (Davignon *et al*., 1999).

Existem 3 alelos em seres humanos para Apo E: E2, E3 e E4. A elevação das concentrações séricas de colesterol e triacilgliceróis é causada pela homozigose do alelo E2 e está associada ao acúmulo de remanescentes de quilomícrons de VLDL no plasma, caracterizando dislipidemia, maior fator de risco para a aterosclerótica (Weisgraber *et al*.,1981, 1982).

Camundongos de laboratório são naturalmente resistentes ao desenvolvimento da aterosclerose em condições normais. Diferente dos humanos em camundongos o transporte do colesterol ocorre via HDL (Kleemann 2007). Concentrações séricas de VLDL e LDL são menores (Plump *et al*., 1992; Zhang *et al*., 1992). A deleção direcionada do gene para a Apo-E provoca um estado que leva a hipercolesterolemia grave e aterosclerose espontânea. O melhor modelo animal para estudo da aterosclerose, e perfil

bioquímico dos lipídios são os camundongos nocautes camundongos nocaute, Apo-E$^{-/-}$ e LDLr$^{-/-}$.

Os camundongos também apresentam a ausência da lipoproteína A, uma lipoproteína pró-aterogênica presente em humanos. Apesar das diferenças no transporte e no metabolismo de lipídios entre camundongos e humanos, modificações em pontos específicos das vias tornam as espécies análogas (Smithies & Maeda, 1995).

Quando tratados a partir de dieta aterogênica por diversas semanas, algumas linhagens de camundongos desenvolveram lesões com estrias de gordura na porção proximal da aorta (Roberts & Thompson, 1976). Nos anos 90, a engenharia genética permitiu o aprimoramento de modelos de camundongos transgênicos que são capazes de desenvolver lesões avançadas quando comparados com as atenuadas injúrias observadas em camundongos selvagens alimentados com dieta aterogênica (Breslow, 1996). Há assentimento de que camundongos Apo-E$^{-/-}$ podem acentuar lesões que se associam a aspectos semelhantes às lesões vasculares humanas (Smithies & Maeda, 1995; Calara *et al*., 2001; Johnson & Jackson, 2001).

2.2 Metabolismo de lipídios e lipoproteínas

Os lipídios constituem os componentes básicos das membranas celulares e a principal forma de estoque energético no organismo humano. Os principais lipídios do plasma humano são colesterol, fosfolipídios, ésteres de colesterol, triacilglicerol e ácidos graxos não-esterificados (Babin et al 2009). Essas biomoléculas são substâncias insolúveis em água, portanto devem ser transportadas por complexos macromoleculares de lipídios e proteínas – as lipoproteínas são divididas de acordo com sua densidade, origem, composição e tamanho em cinco classes: quilomícrons (QM), lipoproteína de densidade muito baixa (VLDL), lipoproteínas de baixa densidade (LDL), lipoproteínas de densidade intermediária (IDL) e lipoproteínas de alta densidade (HDL). As lipoproteínas participam do transporte do colesterol de colesterol e triglicérides (Levinson, 2015) do fígado, que realiza a produção endógena colesterol e do intestino que absorve o colesterol advindos da dieta (exogeno), para os locais de utilização e armazenamento.

As apoproteínas (Apo), que correspondem a parte proteica das lipoproteínas, são um grupo de proteínas que conferem estabilidade às partículas de lipoproteínas, bem como o seu direcionamento metabólico, devido à interação com receptores específicos em células alvo. Além disso, elas atuam como cofatores para as enzimas envolvidas no metabolismo de lipoproteínas. (Mackeness & Durrington, 1995).

Os triglicérides e o colesterol entram no plasma na forma de partículas de lipoproteínas ricas em quilomícrons e VLDL, sofrendo mudanças intravasculares através da enzima lípase lipoproteíca, que hidrolisa os triglicérides e os diglicérides em ácidos graxos e monoglicérides, respectivamente. As apolipoproteínas são transferidas da VLDL à HDL a partir da interação com a lectina-colesterol aciltransferase para a síntese de ésteres de colesterol e lisolectina. Posteriormente, os ésteres de colesterol são transferidos à IDL, que é convertida em LDL ricas em ésteres de colesteril. A LDL é, assim, um produto final do metabolismo da VLDL intravascular (Kang, *et al*., 2002; McEneny *et al*., 2013). O colesterol representa o principal componente da LDL, enquanto representa uma fração menor das VLDL e HDL.

O HDL possui papel importante no transporte reverso de colesterol dos tecidos extra-hepáticos de volta para o fígado, onde é excretado na bile (Fisher *et al* 2013). A remoção do excesso de colesterol celular das paredes tem papel muito importante ao diminuir o acúmulo de colesterol, atenuando o processo da aterosclerose, portanto, considerada como uma lipoproteína anti-aterogênica. O fígado, o intestino e os remanescentes de lipoproteínas são as principais fontes de HDL, cujas principais apoproteínas são Apo A-I e Apo A-II (Fisher *et al* 2013).

2.3 Dieta hiperglicídica

Há muito tempo, pesquisadores têm procurado fazer associações entre padrões alimentares e riscos de morbidade e mortalidade. Com o término da Segunda Guerra Mundial, a abundância na oferta de alimentos e as drásticas mudanças no estilo de vida moderno, contribuíram para uma grande epidemia de obesidade, especialmente nos Estados Unidos, onde mais da metade da população está acima do peso. Isso é previsto para ser um grande problema de saúde pública em um futuro próximo (Neel, 1999).

A partir dos anos 50, trabalhos indicaram pela primeira vez a associação entre concentrações de colesterol sérico e problemas cardíacos (Dawber *et al*., 1957). Surgiram a partir daí as primeiras recomendações, por parte de cientistas e da *American Heart Association*, para diminuição da ingestão de gordura, principalmente a saturada. Com intuído de diminuir as concentrações séricas de lipídios. Além disso, os triglicérides são um fator de risco independente para doenças cardiovasculares e também é um fator de risco para o desenvolvimento de doenças cardíacas. (Austin *et al*., 1998).

A dieta hipolipidica é considerada como saudável (Taubes, 2001). Erroneamente atribui-se o aumento da adiposidade corporal com dietas ricas apenas em gordura e que os alimentos *low-fat* (pobres em gorduras) não seriam capazes de desenvolver acúmulo de gordura corporal. Muitos produtos pobres em gordura possuem alta densidade energética além de serem ricos em carboidratos (Katan, 1998).

No Brasil e em outros países do mundo foram elaboras políticas públicas com a intenção de reduzir o consumo de lipídios, impedindo a progressão da obesidade que é crescente em muitos países (Mendez *et al*., 2005). No Brasil, o perfil atual mostra que 59% das calorias estão representadas por carboidratos; 12%, por proteínas; e 29%, por lipídeos. De modo geral há uma grande ingestão de carboidratos (Santos *et al*., 2013). O Guia Alimentar para a População Brasileira recomenda que o consumo de lipídios esteja em aproximadamente 20%, recomendação similar à aquela proposta pela Organização Mundial de Saúde (WHO, 2003).

Ainda é debate a proposição de qual real benefício das dietas hipolipídicas, como caráter preventivo, indivíduos saudáveis que ainda não apresentam fatores de risco para doenças cardiovasculares (Taubes, 2001). Apesar da redução do consumo de lipídios na dieta as intervenções médicas aumentaram de 1,2 para 5,4 milhões (Taubes, 2001). Isto significa que não houve diminuição na incidência das doenças cardiovasculares, mas, sim, avanços na área médica, bem como métodos mais eficientes e de alto grau de sensibilidade para diagnosticar, mais precocemente, os casos o que pode ter interferido no quadro de mortalidade (Polacow & Lancha Junior, 2007).

A obesidade é um fator de risco, não só para doenças cardiovasculares, mas também para dislipidemias, diabetes *mellitus* tipo 2, hipertensão, osteoartrite, apnéia do sono entre outros, procura-se cada vez mais descobrir

padrões alimentares que predisponham ao ganho de gordura corporal, com o objetivo de implantar políticas de saúde pública de prevenção do problema (Allison & Saunders, 2000).

Ainda que existam recomendações para a adoção de dietas hipolipídicas, a manipulação dietética é mais complexa. Uma vez que se diminui o conteúdo de algum nutriente específico, altera-se toda a composição da dieta. Dietas hipolipídicas, contendo menos do que 30% do valor energético total (VET) na forma de lipídios, são, frequentemente, hiperglicídicas. Enquanto uma restrição moderada em lipídios (25 a 30% do VET) pode resultar em uma dieta normoglicídica, contendo aproximadamente 55% do VET na forma de carboidratos e restrições mais severas (10 a 15% do VET na forma de lipídios) resultam em dietas hiperglicídicas (mais do que 60% do VET na forma de carboidratos). O Guia Alimentar para a População Brasileira, 2005, recomenda que o consumo de carboidratos fique entre 55 e 75% do valor energético total, e o Dietary Guidelines for Americans, 2005, recomenda, entre 45 e 65%.

A principal crítica feita às recomendações para a adoção de dieta hipolipídicas, portanto, é a de que estas encorajariam uma mudança alimentar para dietas ricas em carboidratos, os quais acabam sendo em especial aqueles provenientes de alimentos processados e com alto índice glicêmico, que podem oferecer efeitos adversos graves à saúde, entre os quais estão: hipertrigliceridemia e diminuição na concentração plasmática de HDL (Krauss*et al*., 2000; Astrup, 2000; Dietary Guidelines for Americans 2005) e aumento da adiposidade (Parks, 2001).

A questão chave que merece destaque seria qual o nutriente deve-se utilizar para substituir a gordura saturada já que este é tipo de gordura dietética que está intimamente relacionada com o risco cardiovascular. Diante disso, existem duas estratégias comumente utilizadas e recomendadas para prevenir e combater os riscos de doenças cardiovasculares e a síndrome metabólica, sendo que ambas dizem respeito à composição da dieta (Grundy & Chandalia, 2002).

A primeira estratégia é a substituição isoenergética por carboidratos o que resulta em uma dieta hipolipídica e hiperglicídica. Já que uma substituição por lipídios ricos em ácidos graxos monoinsaturados configuraria uma dieta semelhante à dieta mediterrânea, que e é conceituada por alguns pesquisadores, que consideram a substituição isoenergética de gorduras por

carboidratos um risco à saúde (Estruch *et al*., 2006). Dessa forma, muito se discute a respeito da capacidade que dietas ricas em carboidratos teriam a prevenção de doenças cardiovasculares e obesidade. Ainda é mistério, quanto a substituição de gorduras por carboidratos pode influenciar positivamente e até que circunstância se pode limitar o consumo de lipídios, aumentando a ingestão de carboidratos, sem causar efeitos adversos, por exemplo, no perfil lipídico e na adiposidade corporal (Polacow & Lancha Junior, 2007).

A via enzimática responsável pelo qual carbonos oriundos de carboidratos são convertidos a gorduras (ácidos graxos) recebe o nome de lipogênese *de novo* (Parks, 2002). A relevância existente nesse processo refere-se ao seu possível papel no desenvolvimento de dislipidemias e no acúmulo de gordura corporal.

Fatores relacionados com a dieta podem influenciar, direta ou indiretamente, a expressão de genes que participam da lipogênese *de novo*, como a acetil-CoA carboxilase (ACC), enzima responsável pela carboxilação do acetil-CoA, reação essencial que é vista como a mais importante na regulação da síntese *de novo* de ácidos graxos, e a ácido graxo sintase, que tem como produto final o palmitato. Há indícios de que ambas as dietas, hipolipídicas e hiperglicídicas, estimulam consideravelmente a lipogênese, aumentando a ativação de enzimas lipogênicas (Delzenne *et al*., 2001; Uyeda *et al*., 2002). O estímulo de enzimas lipogênicas pode ser dado por meio de fatores de transcrição, como o SREBP (sterol regulatory binding proteins); a ChREBP (carbohydrate-responsive element-binding protein), ativada em resposta à alta glicemia e ao estímulo do receptor nuclear PPAR-γ (peroxisome proliferator-activated receptor-gamma) (Kersten, 2001; Uyeda *et al*., 2002; Stoeckman & Towle, 2002).

Quando a glicose é convertida a acetil-CoA através da via glicolítica, estimula a lipogênese pelo fato de ser um substrato para tal evento. Além disso, a glicose plasmática estimula a lipogênese agindo no processo de liberação de insulina, um fator hormonal que afeta de maneira potente a lipogênese, aumentando a captação de glicose pelas células adiposas, assim como ativando enzimas glicolíticas e lipogênicas (Kersten, 2001).

O fígado é o principal órgão responsável por converter o excesso de carboidratos adquiridos através da dieta hiperglicídica. Uma dieta rica em carboidratos provoca a ativação de várias enzimas reguladoras da glicólise e

lipogênese (Troiano & Flegal, 1999). O excesso de carboidratos também resulta na ativação pós-traducional, assim como a transcrição de pelo menos 15 genes codificadores das mesmas enzimas-chave envolvidas no processo metabólico dos resíduos glicídicos e a formação de novos lipídios, promovendo, dessa forma, o armazenamento à longo prazo de carboidratos na forma de triglicérides (Goodridge, 1990).

Vários estudos apontam que dietas hiperglicídicas podem provocar hipertrigliceridemia, induzida por carboidratos, contribuir para a aumentar as concentrações de LDL e reduzir as concentrações de HDL plasmático (Katan, 1998; Krauss, 2000; Parks, 2001).

Alguns autores defendem a idéia que a elevação da trigliceridemia ocorra em decorrência do aumento da produção endógena de ácidos graxos, via lipogênese *de novo*, provocando aumento na produção e liberação de VLDL pelo fígado. Além deste processo, os ácidos graxos para a síntese de triglicérides também podem ser derivados do *pool* plasmático de ácidos graxos livres, dos ácidos graxos estocados no próprio fígado e da dieta, que são disponibilizados ao fígado por meio da remoção de remanescentes de quilomícrons (Parks, 2001).

2.4 Aterosclerose e Estresse Oxidativo

Segundo a Sociedade Brasileira de Cardiologia a mortalidade gerada por doenças cardiovasculares diminuiu nos últimos anos, mas vem aumentando em países em desenvolvimento como o Brasil. Entretanto ainda ocorrem cerca de 17,5 milhões de óbitos por doenças cardiovasculares no mundo (WHO, 2015).

Múltiplos fatores biológicos contribuem para a etiologia das doenças cardiovasculares. Estudos epidemiológicos identificaram diversos fatores de risco para aterosclerose, incluindo idade, sexo, hipertensão, aumento de LDL e redução do HDL, colesterol, triglicérides, tabagismo, histórico familiar, obesidade, diabetes *mellitus* e as doenças crônicas inflamatórias preexistentes, como artrite reumatoide (Fruchart *et al*., 2004; Yusuf *et al*., 2004). Esses fatores variam em prevalência e intensidade, e podem ser combinados culminando em aterosclerose severa, suportando o conceito que essa patologia é uma doença multifatorial (Yusuf *et al*., 2004).

A resposta inflamatória da parede vascular esta injúria é considera o fator inicial capaz de gerar aterosclerose. O primeiro passo que precede a formação da lesão aterosclerótica é a ativação ou disfunção endotelial e deposição de LDL na parede arterial. A LDL em seu estado nativo não apresenta propriedades aterogênicas, sendo necessária a modificação oxidativa dessa lipoproteína para que ela se torne altamente lesiva ao endotélio vascular (Witztum e Steinberg, 2001). A células danificadas pelo acumulo de LDLox e pelo estresse oxidativo, recrutam monócitos e linfócitos-T para a parede do vaso (Kovanen & Pentikainen, 2003; Siqueira *et al*., 2006). Na parede arterial, os monócitos se diferenciam em macrófagos e fagocitam a LDL peroxidada no tecido (Madamanchi *et al*., 2005).

A LDL primeiramente sofre oxidação ainda na corrente sanguínea, formando LDL-ox, mas no ambiente pró-oxidante da túnica íntima na artéria em processo de formação da placa fibrosa, ocorre a maior parte da oxidação desta lipoproteína (Kovanen & Pentikainen, 2003). A oxidação que ocorre pela interação entre os radicais livres e lipoproteína, espécies reativas de oxigênio (EROs), que incluem ânion superóxido (O2-), peróxido de hidrogênio (H_2O_2) e o radical hidoxil (OH) e as espécies reativas de nitrogênio (ERNs) é composta pelo oxido nítrico (NO) e peroxinitrito ($ONOO^-$) que reagem com a LDL para formar a LDL-ox (Singh e Jialal, 2006).

Os macrófagos fagocitam a LDL peroxidada presente no tecido (Madamachi *et al*., 2005). O surgimento das células espumosas ocorre, após a fagocitose da LDL-ox pelos macrófagos, onde a LDLox é degradada, o colesterol livre é esterificado. O resultado do grande acúmulo de colesterol e a formação de células espumosas (Steinberg, 1997) que associadas aos leucócitos, formam as estrias de gordura arteriais originando a primeira lesão da aterosclerose (Madamanchi *et al*., 2005)

Os leucócitos secretam fatores de crescimento que induzem a migração e proliferação das células de musculo liso para a camada íntima com concomitante secreção de colágeno, dando origem a lesão intermediária. Mais uma vez, sob o estimulo da LDLox, entre outros, as células do sistema imune local liberam enzimas, citocinas e fatores de crescimento que podem induzir necrose focal podendo evoluir para uma lesão aterosclerótica avançada (Tedgui, 2006).

2.5 Esteroides anabólicos

A testosterona é o principal hormônio androgênico do sexo masculino secretado pelas células intersticiais de *Leydig*, nos testículos, responsável por diversos efeitos no organismo, sendo alguns deles a determinação das características sexuais masculinas primárias e secundárias, o aumento da massa muscular e do peso corpóreo, fechamento epifisário, aumento da densidade óssea, dentre outros efeitos (Frizon *et al*., 2005). Os esteroides anabólicos androgênicos (EAAs) são compostos naturais e sintéticos formados de testosterona e seus derivados, (Shahidi, 2001) que atuam sobre receptores androgênicos (Asuthkar, 2015) produzindo efeitos tanto anabólicos quanto androgênicos, sendo uns mais androgênicos que outros.

Os EAAs possuem em sua estrutura variações químicas classificadas em 17-alfa-alquelados, 17-beta-ésteres e 1-metil esteroide, as quais exibem peculiaridades distintas, mas com os mesmos propósitos. Dentre suas variações mais usadas dividem-se os EAAs em duas classes: orais (17-alfa-alquelados) em forma de comprimidos e injetáveis (17-beta-ésteres). Os EAAs injetáveis são menos nocivos que os orais e devem ser injetados por via intramuscular profunda. Dentre os injetáveis podemos citar alguns de maior aceitação pelos usuários, como decanoato de nandrolona (deca-durabolin), durateston, composto de quatro sintéticos (propionato, fenilpropionato, isocaproato e decanoato de testosterona), cipionato de testosterona (deposteron) e undecilenato de boldenona (equifort), droga exclusivamente veterinária (Peres & Guimarães Neto, 2005).

O efeito anabólico dos EAAs possibilita maior eficiência no desempenho físico promovendo a eritropoiese, o aumento da síntese proteica, crescimento muscular associado a força, reduzindo também o tempo de recuperação pós-treinamento físico (Mottram *et al*., 2000) sendo utilizados para o aumento da força e resistência de caninos, equinos e atletas humanos (Teale & Houghton, 1991). Em função do aumento da massa muscular mediado pela testosterona, atletas acreditam que as doses suprafisiológicas de EAA possam promover efeitos adicionais sobre a composição corporal, como aumento da massa magra e redução das reservas adiposas (American College of Sports Medicine, 1987).

O tratamento médico os EAAs vêm sendo utilizados em diversas condições patológicas relacionadas com às deficiências androgênicas, balanço

nitrogenado negativo, hipogonoidismo, sarcopenia, câncer de mama, osteoporose, distrofia muscular. Os esteroides anabolizantes androgênicos também promovem efeito placebo, psicológico, euforizante, anticatabólico, diminui o cansaço e melhora a síntese proteica. De acordo com Silva *et al*., (2002), os EAAs podem promover o aumento da massa muscular, retenção de nitrogênio, aumento na deposição de cálcio e aumento da queima de gordura (Silva *et al*., 2002).

Os EAAs em doses terapêuticas causam poucos efeitos colaterais. No entanto as doses comumente administradas por usuários são cerca de 10 a 100 vezes maiores do que as doses fisiológicas utilizadas em terapias de reposição hormonal em homens hipogonadais. Além disso o uso de EAA quase sempre é realizado aassociado a outras drogas, o que vem sendo denominado de “empilhamento” (Wu, 1997), aumentando os potenciais efeitos colaterais nocivos relacionados ao uso indiscriminado destas drogas (Payne *et al*., 2004; Hartgens & Kuipers, 2004; Chaves *et al*, 2007; Hoffman *et al*. 2009;). Alguns dos possíveis problemas relacionados ao uso indevido de EAAs incluem: hipertrofia cardíaca patológica, agressividade, hipertrofia prostática, hipertensão arterial, hipercolesterolemia, insônia, hepatoxidade, mudanças no sistema imunológico além de ser induzir a formação de neoplasias (Fineschi *et al* 2000, Turillazzi *et al*., 2011). Deve-se ressaltar que os EAAs, dependendo da dose administrada, pode levar ao óbito.

2.6 Undecilenato de boldenona (Equifort)

O undecilenato de boldenona é um esteroide anabólico que se difere da testosterona pela presença de uma dupla ligação na posição 1-carbono (17 betahidróxi-androsta–1,4dien–3um10 undecanoato) (Stolker *et al*., 2007) e é uma droga exclusivamente de uso veterinário em equinos. No Brasil é encontrada com o nome comercial EQUIFORT® (Ceva, São Paulo, Brasil) numa concentração de 50 mg de undecilenato de boldenona por mililitros (mL). O Equifort é recomendado como tratamento complementar de patologias como: distrofia muscular, osteoporose, e excesso de treinamento. O mecanismo dessa droga aumenta a massa muscular a partir do balanço positivo de nitrogênio, estimulando a produção de proteínas e redução na perda protéica bem como provocando a retenção de íons como nitrogênio, sódio, potássio e

cálcio (Forbes, 1985; Mooradian *et al*., 1987). A boldenona é uma droga bem tolerada por apresentar efeitos colaterais discretos quando administrada de forma adequada apresentando resposta terapêutica imediata e duradoura, com uma meia vida de 14 a 16 dias aproximadamente, apresentando propriedades anabólicas acentuadas, pouca atividade androgênica (por apresentar pouca afinidade com a enzima 5-alfa-reductase), hepatoxidade moderada e baixa aromatização (por ter pouca afinidade com a enzima aromatase) (EQUIFORT, 1996; Quel, 2015).

Similar a outros esteroides androgênicos, o undecilenato de boldenona é enquadrado como classe 2ª (promotores de crescimento – esteroides) conforme a Agência Internacional de Pesquisa sobre o Câncer sendo um provável agente carcinogênico humano, com índices de cancinogenicidade maiores que de outros andrógenos como nandrolona e estanozolol, sendo portanto, uma substância proibida (IARC, 1987). Apesar das restrições, os EAAs são facilmente vendidos e o uso indiscriminado deste esteroide pode levar a danos graves aos órgãos de forma irreversível (Maravelias, 2005). Entre os efeitos adversos mais comuns dos EEAs descritos pode-se citar: redução da fertilidade (Dohle, 2003), hipertensão (Ferenchick, 1990), aterosclerose (Cohen *et al*., 1988), formação de coágulos sanguíneos (Pärssinen & Seppälä, 2002), neoplasias hepáticas e carcinoma (Velazquez & Alter, 2004). Se administrado de forma incorreta pode ocasionar efeitos androgênicos tais como: aumento da oleosidade cutânea, queda de cabelo, aumento da pressão arterial, hepatoxidade, ginecomastia (Quel, 2015).

A frequência com que baixos níveis de testosterona são encontrados em pacientes com aterosclerose (Wu & Von Eckardstein, 2003; Traish *et al*., 2009; Akishita *et al*., 2010) torna a concentração deste hormônio preditora desta patologia (Fadini *et al*., 2009; Kaushik *et al*., 2010).

O excesso de testosterona exógena pode ser convertido em estrógeno via aromatase, que possui efeito sobre a permeabilidade e função vascular. Os estrógenos podem ainda exercer influência sobre mensageiros endoteliais como o óxido nítrico (NO^-), que possui ação vasodilatadora, aumentando a atividade da oxido nítrico sintetase responsável pela produção do NO^- endotelial (Mendelsohn, 2000). O NO^- é considerado um radical livre antiaterosclerótico por inibir a adesão de monócitos às células endoteliais,

inibição da proliferação de células de músculo liso, além de sua ação como vasodilatador e inibidor da agregação plaquetária (Vanhoutte, 2009).

Existem poucos estudos que investigaram os efeitos da administração do esteroide, undecilenato de boldenona, sobre os parâmetros morfológicos e bioquímicos do tecido cardíaco de animais ateroscleróticos. Assim sendo, o presente estudo tem como objetivo elucidar o efeito da administração de undecilenato de boldenona sobre o perfil lipídico e de citocinas, morfologia, atividade das enzimas antioxidantes do tecido cardíaco de camundongos Apo-$E^{-/-}$ tratados ou não com dieta hiperglicídica.

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ARTIGO

High carbohydrate dietary and Boldenone undecylenate independently modulates lipid metabolism, cytokines profile and heart morphology in APOE$^{-/-}$ mice

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Abstract

In this study we investigated the effects of high-carbohidrate dietary (HC) intake and boldenone undecylenate (Bol) on lipid and cytokine profiles, redox balance and heart morphology in Apo-E$^{-/-}$ dyslipidemic mice. Female mice, were randomized in 6 group with 8 animals each: Control) C57BL/6 + standard diet; NT) Apo-E$^{-/-}$ + standard diet; Bol) Apo-E$^{-/-}$ + standard dietary + 20 mg/kg Bol; HC) Apo-E$^{-/-}$ + HC; Bol 10 and Bol 20) Apo-E$^{-/-}$ + HSD + 10 or 20mg/kg BOL, respectively. The HSD was developed according the protocol AIN-93G for purified diets. Bol was administered intraperitoneally three times per week, for 8 weeks. Our data indicated that HC and Bol alone or combined increased body weight, LDL and HDL cholesterol serum levels, and cardiac activity of glutathione-s-transferase in Apo-E$^{-/-}$ mice. Conversely, animals treated with HC or Bol alone presented reduced activities of catalase and superoxide dismutase. All groups receiving HC or Bol presented reduced tissue levels of malondialdehyde. CK-MB levels were high in animals treated with BOL or HSD alone, but not combined. In general, HSD increased TNF-α, IFN-γ, IL-2, IL-10 and IL-17 serum levels. However, this effect was variable in animals receiving HSD plus BOL. Although HSD and BOL have not exercised detectable influence on the morphology of the cardiac parenchyma, tissue vascularization was markedly increased by both treatments, especially when combined and in a dose-dependent way. While HC alone increased myocardial accumulation of inflammatory cells, this effect was attenuated by Bol. Taken together, our findings indicated that both HC and Bol can independently aggravated the dyslipidemia and induce a marked vascular remodeling in cardiac tissue. Apparently, HC is potentially more dangerous that Bol, inducing a marked pro-inflammatory systemic status, which is potentially related to the cardiac inflammatory infiltrate in Apo-E$^{-/-}$ mice. Although HC and Bol can impair the activities of antioxidant enzymes, this effect seem to be not harmful to the cardiac redox balance.

1 Introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and death worldwide. Word Health Organization estimate that 17.5 million people died from CVDs in 2012, representing 31% of all global deaths. Of these deaths, an estimated 7.4 million were due to coronary heart disease and 6.7 million were due to stroke (WHO, 2015). Atherosclerosis is the most important cardiovascular disease (Libby 2006). This disease has a multifactorial origin (Yusuf *et al*., 2004), characterized by lipid infiltration and chronic inflammation of the vessel wall (Ross 1999; Elena & Klaus 2009).

Low density lipoprotein (LDL) is considered a pro-atherogenic lipoprotein (St-Pierre *et al*., 2001; Libby, 2006; Hansson *et al*., 2006). Elevated levels of this lipoprotein in plasma leading a pro-inflammatory reaction (Libby 2002; Altman, 2003) and disrupted the redox balance, triggering the atherosclerosis (Stocker *et al*., 2007; Zhang & Gutterman, 2007). It is known that oxidative stress (OS) and inflammation play the central role in the development and progression of atherosclerosis. In addition, the cardiovascular risk factors such as hypertension, hypercholesterolemia and dyslipidemia, which increase the progression of atherosclerosis, also increase oxidative stress levels (Stephens *et al*., 2006; Chaves *et al*., 2007; Hervas *et al*., 2008). Although not well known, anabolic androgenic steroids (AAS) has a pro-atherogenic effect when administered in supraphisiological doses (Fogarty *et al*., 2004; Basaria *et al*., 2010), besides modify the lipid profile and increase oxidative stress.

AAS are synthetic derivatives of testosterone that have been modified to improve their anabolic and androgenic effect. Boldenone undecylenate (1,4-androstadiene-17β-ol-3-one) (Bol) is an anabolic androgenic synthetic commercial steroid (Soma *et al*., 2007 Cannizzo *et al*., 2007; Tousson *et al*., 2016) sold by the name Equiforte (Ceva©, São Paulo, Brasil). It was developed just for veterinary use, mainly for the horse coadjuvant treatment in muscular dystrophy, osteoporosis, and anemia. Moreover, Bol is also indicated to overtraining and post-surgical treatments (Euiforte, 1996). However, to enhance performance, strength and even for improving the physical appearance and body image, the use of Bol by people is growing. (Copeland *et al*., 2000; Larance *et al*., 2008; Petersson *et al*., 2010; Hakansson *et al*., 2012; Darke *et al*., 2014).

Historically, in the 1950s, it was found in the United States, an association between increased serum cholesterol levels and heart problems. As a result of this association were created guidelines for reduce the intake of fat, particularly saturated, for individuals who have higher concentrations of cholesterol. One of the strategies commonly used to prevent and combat cardiovascular risks is the low fat dietary. However, this dietary is rich in carbohydrates (Polacow 2007). It is known that carbohydrate dietary (HC) may induce hypertriglyceridemia by the higher levels of carbohydrates, rising the formation of LDL particles and reducing high density lipoprotein (HDL) cholesterol (Graham *et al*., 2010 Sirjani *et al*., 2014).

Although there are many studies regarding the high-carbohydrate dietary or the use of anabolic steroids, no apparent study was found about the correlation between these factors, mainly in cardiac tissue. Therefore, the objective of this study was investigate the effects of boldenone undecylenate and high carbohydrate dietary in cardiac tissue of murine model of atherosclerosis. Our results suggest that treatment whit Bol and HC alter the lipid profile and the redox balance, and induce a pro-inflammatory status.

2 Material and methods

2.1 Experimental protocol

Forty female knockout Apo-E$^{-/-}$ mice and eight female C57BL/6 wild type (age, 10 weeks;) were obtained from the Central Animal Laboratory of the Center of Biosciences and Health of Federal University of Viçosa (Brazil). The animals were maintained under conditions of controlled temperature at 21 ± 2℃, relative humidity of 60% to 70%, and 12-h light/dark cycles. The food and water were provided *ad libitum*. All protocols were approved by the Ethics Committee of Animal Use of the Federal University of Viçosa (CEUA/UFV, protocol 35/2015) and carried out in compliance with the guidelines issued by the National Council for the Control of Animal Experimentation (CONCEA).

2.2 Treatment

The animals were randomly divided in 6 groups. The control group was composed by C57BL/6 mice. The following treatments were performed on Apo-

$E^{-/-}$ mice. NT (nontreated); BoL (boldenone 20 mg/kg body weight); HC (high carbohydrate dietary); BoL 10 (boldenone 10 mg/kg body weight and high carbohydrate dietary); BoL 20 (boldenone 20 mg/kg body weight and high carbohydrate dietary). The high carbohydrate dietary followed the propose of the American Institute of Nutrition (AIN-93M), according to Reeves, 1997. (Table 1). Boldenone undecylenate (Ceva®, São Paulo, Brazil) was dissolved in vegetable oil and administered intraperitoneally three times per week. All treatments were administered for 8 weeks. The animals were euthanized 24 h after the last treatment by deep anesthesia (ketamine 45 mg/kg and xylazine5 mg/kg, i.p.), followed by cardiac puncture. After thoracotomy, blood samples were collected and centrifuged at 3.000 × g for 15 min for obtaining serum. Serum was collected to lipid profile analysis, determination of biomarkers to cardiomyocytes damage and determination of cytokines levels. The heart was removed, weighed, and the cardiosomatic index (CSI, %) was calculated by normalizing the heart weight to the final body weight.

Table 1. Composition of normoglycidic and high carbohydrate dietary

Composition	Standard	High carbohydrate dietary
Casein (100%)	20	14
Sucrose	10	60
Maltodextrine	13.2	0
Fiber	5	5
Starch	42.87	12.07
Mineral Mix	3,5	3.5
Vitaminic Mix	1	1
L-cistina	0.18	0.18
Bitartrate choline	0.25	0.25
Soy oil	4	4

Data are expressed as percentage (%).

2.3 Biochemistry profile of lipids

The high density cholesterol, low density cholesterol, triacylglycerol and total cholesterol were measured in the serum using a commercial diagnostic kit (BioClin, Belo Horizonte, Minas Gerais, Brazil) and automatic analyzer (Cobas®, Clinical Laboratory of Health Division of the Federal University of Viçosa, Brazil).

2.4 Antioxidant enzymes assay

Activity assay of catalase (CAT), glutathione s-transferase (GST), and superoxide dismutase (SOD) were performed with the supernatant obtained from the following: an aliquot of frozen cardiac muscle (50 mg) was homogenized in ice-cold phosphate buffer (pH 7.0) and centrifuged at 4000 g (5℃) for 10 min . CAT activity was evaluated according to the method described by Aebi (1984) by measuring the kinetic of the decomposition of hydrogen peroxide (H_2O_2). GST activity was followed spectrophotometrically at 340 nm as described by Habig *et al*. (1974) and calculated from the rate of NADPH oxidation. SOD activity was estimated by pyrogallol method based on the ability of this enzyme to catalyze the reaction of the superoxide (O_2) and hydrogen peroxide (Dieterich *et al*., 2000).

2.5 Lipid and protein oxidation

For analysis of tissue malondialdehyde (MDA), an end product of lipid peroxidation, 100 mg of frozen heart was homogenized in phosphate buffer (pH 7.0) following by centrifugation at 10000 xg for 10 min. The supernatant was reacted with thiobarbituric acid solution (trichloacetic acid 15%, thiobarbituric acid 0.375%, and 0.25 N HCl) for 15 min. The formation of thiobarbituric acid-reactive substances was monitored at 535 nm as described previously (Buege & Aust 1978).

The total protein levels in the heart tissues were determined by using the Bradford method (Bradford 1976). Protein carbonyl content was measured biochemically in the cardiac tissue pellets by adding 0.5 ml of 10 mM 2,4-dinitrophenylhydrazine (DNPH). The reaction involved derivatization of the carbonyl group with DNPH, leading to the formation of a stable 2,4-dinitrophenyl (DNP) hydrazone product, which indicates the content of protein carbonyl in the samples. The optical density (OD) was measured spectrophotometrically at 370 nm (Levine, 1990).

2.6 Cardiomyocyte damage

Cardiomyocyte damage was investigated by spectrophotometric quantification of creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH)

serum levels by using a commercial diagnostic kit (BioClin, Belo Horizonte, Minas Gerais, Brazil).

2.7 Cytometric bead array for cytokines

Analysis was conducted using a BD cytometric bead array mouse th1/th2 cytokine kit (Cytometric bead array [CBA]; BD Biosciences, San Diego, CA, USA). The data were collected using FACSVerse and analyzed with FCAP 3.0 software according to the manufacturer instructions. The following cytokines were measured in serum: tumor necrosis factor alpha (TNF-α), gamma interferon (IFN-γ), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-17 (IL-17) and interleukin-10 (IL-10).

2.8 Sample size and tissue processing

The hearts from five animals per group were removed for stereological analysis. The sample size for this analysis was determined considering the probability P=1/2 for the increase or decrease of the variables of interest. Thus, considering the significance level α=0.05, the minimal significant number of animals used in the statistical analysis was $P=(1/2)^{events}$; so, if n=5, $P=(1/2)^5$ or P=0.03; thus, P<0.05 (Novaes *et al*., 2013).

The left ventricle (LV) was dissected and placed into histological fixative for 48 hours (freshly prepared 10% w/v formaldehyde in 0.1M phosphate buffer pH 7.2). The entire LV was dehydrated in ethanol series, cleared in xylene and embedded in plastic resin. Blocks were cut into 3 µm-thick histological sections, stained with hematoxylin-eosin (H&E), and mounted on histology slides. To avoid repeated analysis of the same histological area, sections were evaluated in semi-series, using one in every 20 sections. The slides were visualized and the images captured using a light microscope (Olympus BX-60®, Tokyo, Japan) connected to a digital camera (Olympus QColor-3®, Tokyo, Japan) (Novaes *et al*., 2011).

2.9 Stereological analysis

All of the stereological analysis was performed according to Brüel *et al*. (2005). The volume density occupied by cardiomyocytes (Vv[*cmy*], %),

interstitium (Vv[*int*], %) and blood vessels (Vv[bvs], %) was estimated by point counting according to the following formula:

$$\mathrm{Vv}[structure\ /\ \mathrm{LV}] = \frac{\sum P[structure]}{\sum Pt};$$

(1)

where Σ*P*[structure] is the number of points that hit the interest structure and Σ*Pt* denotes the total test points. For these analyses, a test system of 42 points was used in an unbiased two-dimensional test area (*At*) of $1.38\times10^{4}\mu m^{2}$ at tissue level.

The length density of the cardiomyocytes (Lv[cmy], mm^{-2}) and blood vessels (Lv[bvs], mm^{-2}) were estimated as follows:

$$\mathrm{Lv}[structure\ /\ \mathrm{LV}] = 2 \times \frac{\sum Q^{-}[structure]}{\sum P[RA;LV]} \times \frac{Pt}{At};$$

(2)

where ΣQ^{-}[*structure*] denotes the total number of interest structure profiles counted in the *At*, and Σ*P*[*LV*] is the total number of points hitting the RA and LV (the reference space) (Brüel *et al*., 2005).

The surface area density of the cardiomyocytes (Sv[cmy], mm^{-1}) and blood vessels (Sv[bvs], mm^{-1}) were estimated using the following equation:

$$\mathrm{Sv}[structure\ /\ \mathrm{LV}] = 2 \times \frac{\sum \mathrm{I}[structure]}{\sum P[structure]} \times \frac{Pt}{l};$$

(3)

where Σ*I*[*structure*] denotes the total number of intersections between the test lines (here 21) and the surface area of the structure of interest, and l is the length of a test line.

The mean diffusion distance from capillary to tissue ($\hat{r}$[bvs], μm^{2}), was obtained from equation 4:

$$\hat{\mathrm{r}}[bvs\ /\ \mathrm{RA;LV}] = \sqrt{\frac{1}{\pi \times \mathrm{Lv}[bvs]}};$$

(4)

The mean cross-sectional area of cardiomyocytes (ā[cmy], μm^2) was calculated as follows:

$$\bar{a}[cmy] = \frac{Vv[cmy]}{Lv[cmy]};$$

(5)

The number density of interstitial cells (Q_A[Ic], cell/mm^2) was analyzed from the cell counting in a standardized test area (At= $1.38\times10^4 \mu m^2$) as follows (Mandarim-de-Lacerda, 2003):

$$Q_A\,[Ic] = \frac{\sum Ic}{At}$$

(6)

For all of these analyses, sixty microscopic fields (magnification ×400) were randomly sampled and a total of $8.27 \times 10^5 \mu m^2$ of myocardium area was analyzed for each group.

2.10 Statistical analysis

Data were presented as mean and standard deviation of the mean (mean ± SD) or median and interquartile range. The normality in data distribution was verified using the D'Agostinos-Pearson test. All biometrical and stereological data were compared using one-way ANOVA, followed by Student-Newman-Keuls *post-hoc* test. A probability of $p < 0.05$ was considered statistically significant. Nonparametric data were analyzed by Kruskal-Wallis test comparing the means by Dunn's test. Differences were considered significant when $p < 0.05$.

3 Results

3.1 Body weight and cardiosomatic index

Final body weight and cardiosomatic index are shown in table 2. The groups NT, Bol, HC, Bol 10 and Bol 20 showed a significant increase in body weight compared to the control mice group. In addition, all animals that received treatment increased significantly the body weight compare to NT mice. Moreover, Bol group showed the higher body weight compared to all groups analyzed ($p < 0.05$). The cardiosomatic index increased significantly in animals

treated with lowest dose of boldenone, 10 mg/kg, and fed com high carbohydrate dietary (Bol 10) in relation to other groups.

3.2 Lipid Profile

Plasma samples were collected to examine the lipid profile differences among the groups. Table 2 shows serum levels of total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides (Tri). TC levels were significantly higher in all groups compared to the control group. In addition, Apo-$E^{-/-}$ mice that received treatment showed higher levels of TC than Apo-$E^{-/-}$ NT mice. Besides, HDL serum levels were significantly reduced in all treatments when compared to Control. In addition, BoL and BoL 10 showed lower HDL serum levels compared to the others Apo-$E^{-/-}$ mice group ($p < 0.05$). Levels of LDL increased significantly in all Apo-$E^{-/-}$ mice. However, Apo-$E^{-/-}$ mice treated showed higher LDL levels than NT ($p < 0.05$). The serum levels of Tri increased significantly in NT, Bol and Bol 10 compared to the Control mice. The group treated with Bol alone increased levels of Tri compared to others treatments.

Table 2 Lipid profile of mice treated with boldenone, high carbohydrate dietary or differents combinations.

	Control	NT	Bol	HC	Bol 10	Bol 20
Weight	17.33±1.12^{a}	25.98±2.14^{c}	30.11±1.61^{d}	23.26±1.25^{b}	24.67±2.00$^{b.c}$	24.56±1.034$^{b.c}$
CSI	0.5982±0.064^{a}	0.5520±0.089^{a}	0.5731±0.07^{a}	0.5675±0.05^{a}	0.7188±0.12^{b}	0.6416±0.05$^{a.b}$
TC	114.0±13.95^{a}	202±22.86^{b}	328.5±15.61^{c}	311±58.97^{c}	320±27.44^{c}	313±22.53^{c}
HDL	43.67±4.1^{a}	29.5±6.4^{b}	21.0±2.0^{c}	31.67±1.24^{b}	21.5±3.78^{c}	29.67±4.49^{b}
LDL	58.93±9.32^{a}	155.4±18.53^{b}	279.9±14.31^{c}	268.0±52.87^{c}	279.0±21.71^{c}	255.2±23.5^{c}
TRI	67.0±11.56^{a}	104.5±23.78^{b}	138.0±28.32^{c}	77.5±9.48$^{a.b}$	100.0±39.45^{b}	92.67±4.55$^{a.b}$

Data are expressed as means ± SD. Different letters in the same row (a, b and c) denote statistical difference among the groups ($p < 0.05$). Cardiosomatic index (CSI), Total cholesterol (TC), High density lipoprotein (HDL), Low density lipoprotein (LDL), Triacylglycerol (TRI). Control; C57BL/6 mice, following treatments were performed on Apo-E $^{-/-}$ mice, NT; mice nontreated, Bol; boldenone 20 mg/kg, HC; carbohydrate dietary, Bol 10 and 20; lowest dose (10 mg/kg) and higher dose (20 mg/kg) both associated to high carbohydrate dietary. The doses of boldenone were administered 3 times per week.

3.3 Activity of antioxidant enzymes

Animals that received boldenone alone (Bol) and high carbohydrate dietary (HC) showed reduction in the activity of catalase (CAT), in heart tissue, compared to other treatments. Moreover, Bol was the group that showed the lower activity of this enzyme ($p < 0.05$). The treatment with Bol alone also decreased superoxide dismutase (SOD) activity in heart tissue, while the treatment with the lowest dose (Bol 10) increased SOD activity. The other treatments did not differ among them. Glutathione S-Transferase (GST) activity increased significantly in all treatments compared to the nontreated group (NT) (Fig. 1).

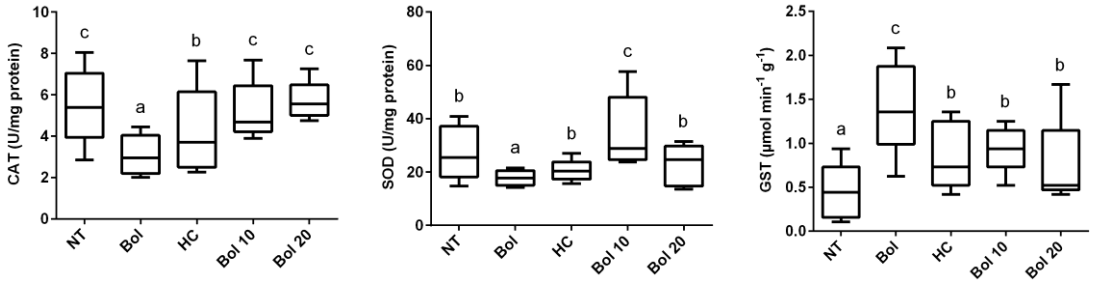

Figure 1. Activity of heart antioxidant enzymes in mice treated with boldenone (Bol) at 10 or 20 mg/kg body weight and fed with high carbohydrate dietary (HC) for a period of 8 weeks. Catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST). NT; nontreated, Bol; boldenone 20 mg/kg, HC; carbohydrate dietary, Bol 10 and 20; lowest dose (10 mg/kg) and higher dose (20 mg/kg) both associated to high carbohydrate dietary. The doses of boldenone was administered 3 times per week. The box represents the interquartile interval with the median indicated (horizontal line), and the whiskers represent the superior and inferior quartiles. Different letters (a, b, c) denote statistical difference among the groups ($P < 0.05$), and groups with common letters do not differ statistically ($P > 0.05$).

3.4 Lipid and protein oxidation

Marker of end products of lipid peroxidation, malondialdehyde (MDA), decreased significantly in all treatments compared to the nontreated group (NT). However, marker of reactive stress by protein oxidation, carbonyl protein (PCN), decreased only in the lowest dose (Bol 10) compared to all groups treated and nontreated ($p < 0.05$). The other treatments did not differ among them (Fig. 2).

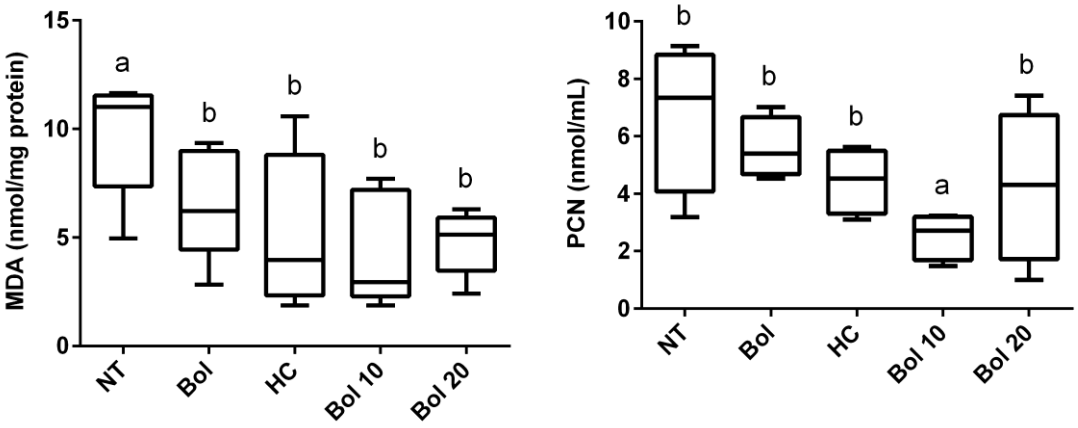

Figure 2. Markers of reactive stress in the cardiac tissue from mice treated with boldenone (Bol) at 10 or 20 mg/kg body weight and fed with high carbohydrate dietary (HC) for a period of 8 weeks. Tissue content of MDA (malondialdehyde) and protein carbonyl (PCN) were used as markers of lipid and protein oxidation, respectively. NT; mice nontreated, Bol; boldenone 20 mg/kg, HC; carbohydrate dietary, Bol 10 and 20; lowest dose (10 mg/kg) and higher dose (20 mg/kg) both associated to high carbohydrate dietary. The doses of boldenone was administered 3 times per week. The box represents the interquartile interval with the median indicated (horizontal line), and the whiskers represent the superior and inferior quartiles. Different letters (a, b, c) denote statistical difference among the groups ($P < 0.05$), and groups with common letters do not differ statistically ($P > 0.05$).

3.5 Cardiomyocyte damage

Creatine kinase-isoenzyme MB (CK- MB) and lactate dehydrogenase (LDH) are used as markers from cardiac damaged. Bol and HC increased the serum levels of CK-MB in these groups compared to the other treatments ($p < 0.05$). The animals exposed to Bol had increase LDH serum levels than other treatments. The other treatments did not differ among them ($p < 0.05$, Fig. 3).

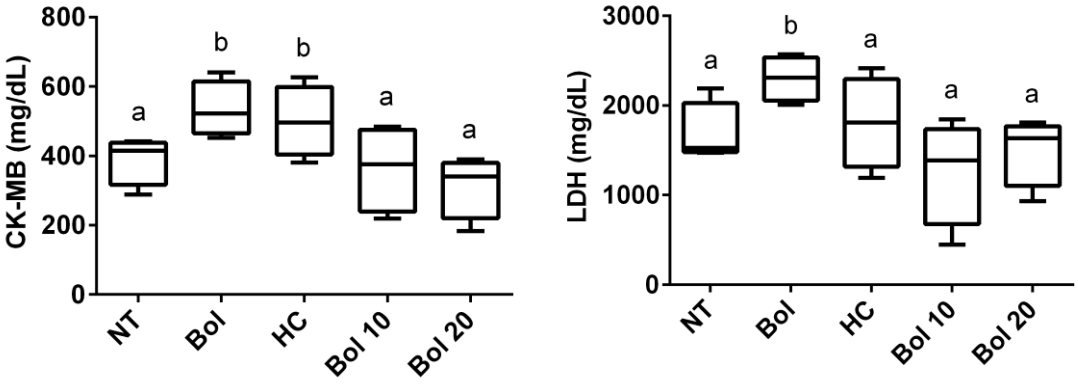

Figure 3. Serum levels of creatine kinase-izoenzyme MB (CK-MB) and lactate dehydrogenase (LDH) in APOE$^{-/-}$ mice treated with boldenone (Bol) at 10 or 20 mg/kg body weight and fed with high carbohydrate dietary (HC) for a period of 8 weeks. NT; mice nontreated, Bol; boldenone 20 mg/kg, HC; carbohydrate dietary, Bol 10 and 20; lowest dose (10 mg/kg) and higher dose (20 mg/kg) both associated to high carbohydrate dietary. The doses of boldenone were administered 3 times per week. The box represents the interquartile interval with the median indicated (horizontal line), and the whiskers represent the superior and inferior quartiles. Different letters (a, b, c) denote statistical difference among the groups ($P < 0.05$), and groups with common letters do not differ statistically ($P > 0.05$)

3.6 Cytokines serum levels

Figure 5 shows the serum levels of the following cytokines, IL-2 TNF-α, IL-6, IFN- γ, IL-17, IL-10. Treatment with high carbohydrate dietary alone (HC) increased significantly the concentration of TNFα in relation to other groups. Animals that received higher and lower dose of boldenone, and animals treated with high carbohydrate dietary alone showed increase in INF-γ ($p < 0.05$) compared to nontreated group (NT). Boldenone alone and the lowest dose of boldenone increased IL6 serum levels. Animals that received high carbohydrate dietary alone showed increased levels of IL-10. However, the other treatments showed decrease in IL-10 serum levels compared to control. IL17 levels also increased in the groups that received boldenone alone and higher dose of boldenone compared to the NT group. The other groups did not show statistical differences. Levels of IL-2 increased in all treatments compared to the NT group, except in the lowest dose treatment, Bol 10.

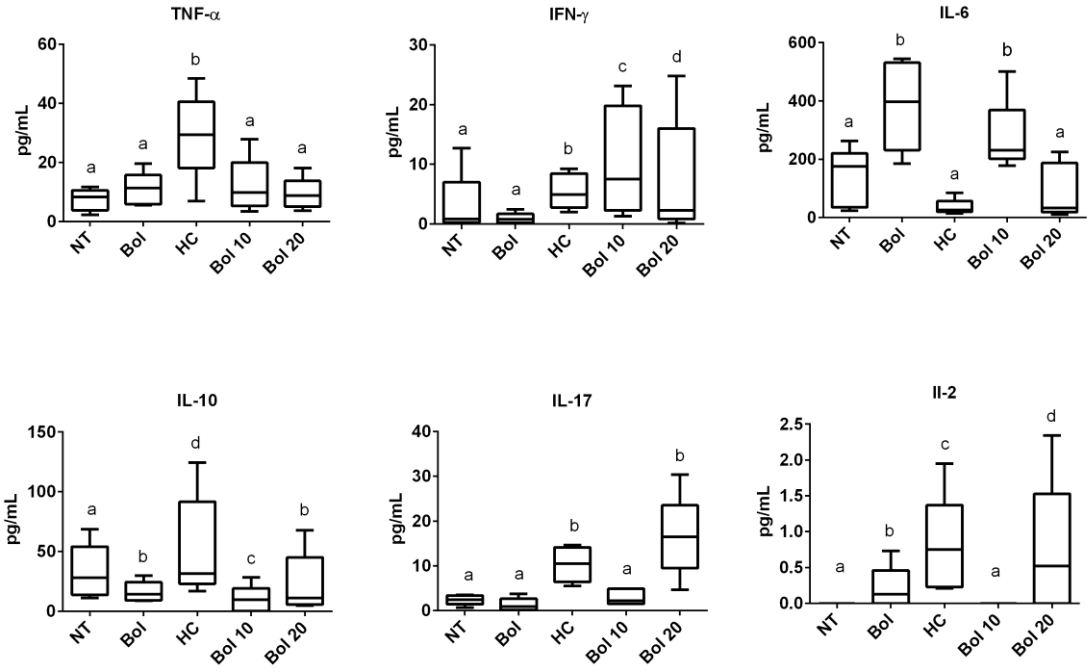

Figure 5. Serum levels of TNf-α, INF-γ, IL-6, IL-10, IL-17 and IL-2 of mice treated with boldenone (Bol) at 10 or 20 mg/kg body weight and fed with high carbohydrate dietary (HC) for a period of 8 weeks. NT; mice nontreated, Bol; boldenone 20 mg/kg, HC; carbohydrate dietary, Bol 10 and 20; lowest dose (10 mg/kg) and higher dose (20 mg/kg) both associated to high carbohydrate dietary. The doses of boldenone was administered 3 times per week. The box represents the interquartile interval with the median indicated (horizontal line), and the whiskers represent the superior and inferior quartiles. Different letters (a, b, c) denote statistical difference among the groups ($P < 0.05$), and groups with common letters do not differ statistically ($P > 0.05$).

3.7 Stereological analysis

Analysis of the stereology of the cardiac tissue showed no differences in the volume density of cardiomyocyte and interstitium. ($p > 0.05$). However, volume density was significantly higher in groups treated with Bol alone, lowest and higher doses. The analysis of the volumetric proportion between blood vessel/cardiomyocyte and the analysis of length density of blood vessel showed increase in all treatments ($p < 0.05$). The mean diffusion distance from capillary to tissue decreased in all treatments compared to nontreated group ($p < 0.05$). No changes were found in surface area density of the cardiomyocytes and mean cross-sectional area of cardiomyocytes ($p > 0.05$). However, the surface area density of the blood vessel increased in all treatments ($p < 0.05$). Presence of inflammatory infiltrate was observed in the groups treated with high carbohydrate dietary alone (HC) and in the group that received the lowest dose of boldenone (Table 3).

Table 3. Stereological data of mice treated with boldenone at 10 or 20 mg/kg body weight and fed with high carbohydrate dietary for a period of 8 weeks.

	Control	NT	Bol	HC	Bol 10	Bol 20
Volume Density						
Cardiomyocyte	79.80±4.13[a]	80.31±2.91 [a]	78.93±2.07 [a]	77.50±3.03 [a]	79.96±4.67 [a]	78.82±2.63 [a]
Interstitium	20.20±4.13 [a]	19.69±2.91 [a]	21.07±2.07 [a]	22.50±3.03 [a]	20.04±4.67 [a]	21.18±2.63 [a]
Blood vessel	5.306±1.50 [a]	4.769±1.35 [a]	9.331±1.35[c]	7.550±1.26[b]	9.953±2.07[c]	10.86±1.64[c]
Vv[bvs]/[cmy]	0.0662±0.017 [a]	0.0593±0.016 [a]	0.1241±0.016[b.c]	0.0979±0.0191[b]	0.1253±0.0295[c]	0.1381±0.0235[c]
Vv[int]/[cmy]	0.2561±0.06 [a]	0.2466±0.044 [a]	0.2677±0.033 [a]	0.2920±0.050 [a]	0.2542±0.072 [a]	0.2699±0.042 [a]
Length density						
Cardiomyocyte	0.065±0.0658 [a]	0.065±0.0657 [a]	0.066±0.0668 [a]	0.066±0.0662 [a]	0.064±0.0644 [a]	0.068±0.0685 [a]
Blood vessel	0.014±0.0018 [a]	0.013±0.0017 [a]	0.023±0.0029[c]	0.016±0.0022[b]	0.021±0.0025[c]	0.024±0.0017[c]
Area	568.6±7.492 [a]	572.8±8.468 [a]	570.6±7.727 [a]	573.8±6.079 [a]	574.4±7.172 [a]	576.1±8.786 [a]
Distance	4.765±0.3129[a]	4.818±0.3138[a]	3.705±0.2328[b]	4.650±0.3425[c]	3.829±0.2337[b]	3.644±0.1342[b]
Surface						
Cardiomyocyte	79.52±3.220 [a]	81.05±3.220 [a]	81.51±3.169 [a]	81.94±4.411 [a]	79.67±2.373 [a]	82.93±3.524 [a]
Blood vessel	10.91±1.815 [a]	11.03±1.453 [a]	15.35±3.078[b.c]	13.54±1.854[b]	18.58±1.929[c]	20.18±2.846[c]
Inflammatory cells	162.9±52.82 [a]	190.0±46.19 [a]	172.9±30.39 [a]	351.4±46.34[c]	272.9±34.98[b]	192.9±44.61[a]

Control; C57BL/6 mice, following treatments were performed on Apo-E -/- mice, NT; mice nontreated, Bol; boldenone 20 mg/kg, HC; carbohydrate dietary, Bol 10 and 20; lowest dose (10 mg/kg) and higher dose (20 mg/kg) both associated to high carbohydrate dietary. The doses of boldenone was administered 3 times per week. Data are expressed as means ± sd. Different letters in the same row (a, b and c) denote statistical difference among the groups ($P < 0.05$)

3.8 Histopathology

The histopathology of the cardiac tissue showed an occurrence of inflammatory infiltrate with a predominance of mononuclear cells in HC and Bol animals, which characterize the chronic inflammatory process (Fig. 6). The control group and nontreated showed regular histopathology.

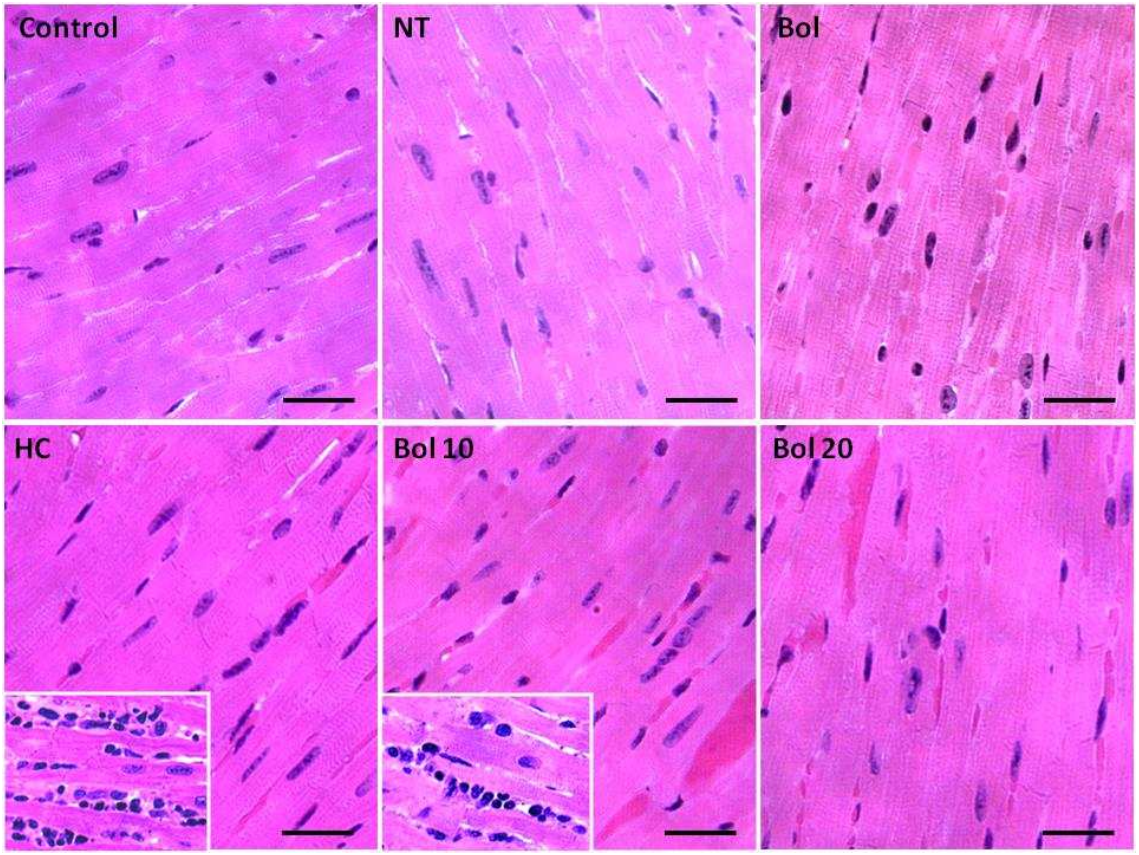

Figure 6. Representative photomicrography's of the cardiac tissue from wild type and APOE$^{-/-}$ mice treated with boldenone (Bol) at 10 or 20 mg/kg body weight and fed with high carbohydrate dietary (HC) for a period of 8 weeks (H&E staining, scale bar = 70 µm). Groups HC and Bol 10 showed leukocyte infiltrate (detail). NT; mice nontreated, Bol; boldenone 20 mg/kg, HC; carbohydrate dietary, Bol 10 and 20; lowest dose (10 mg/kg) and higher dose (20 mg/kg) both associated to high carbohydrate dietary. The doses of boldenone was administered 3 times per week

4 Discussion

The present study investigated the association between high carbohydrate dietary intake and boldenone undecylenate (Bol) in Apo-E mice. The pathogenesis of atherosclerosis involves prolonged exposure to risk factors such as hypertension, higher levels of low density lipoprotein (LDL) and triacylglycerol (TRI), and lowest levels of high density lipoprotein (HDL), (Fruchart *et al*., 2004; Yusuf *et al*., 2004). Increased levels of LDL and TRI characterize the dyslipidemia, the most potent risk factor for atherosclerosis (Talayero *et al*., 2011). Basaria and Severo report that the use of androgenic anabolic steroids (AAS) change the endothelial function, increasing the atherogenic risk. Oxidative stress and inflammation are decisively involved in the initiation and progression of atherosclerosis (del-Ríncon *et al*., 2015).

Our data show that treatments with boldenone alone (Bol), high carbohydrate dietary alone (HC), and also the association between high carbohydrate with anabolic steroid at 10mg (Bol 10) and 20mg (Bol 20), modified the lipid profile in Apo-$E^{-/-}$ mice that received treatments.

The group treated with boldenone had increased LDL cholesterol, triacylglycerol and total cholesterol at the same time that HDL cholesterol was reduced. In addition, the steroid disrupted the redox balance and induced to pro inflammatory status. Have been documented in several studies that AAS decrease HDL (Thompson *et al*., 1989; Bagatell and Bremner *et al*., 1996; van Amsterdam 2010). The reduction of HDL in serum levels is mediated by increase of hepatic triacylglycerol lipase. This enzyme is responsible for regulate HDL metabolism, and for the conversion of very low density lipoprotein (VLDL) to LDL (Ansell JE 1993; Hartgens, 2004; Urhausen, 2004). Besides, high-carbohydrate diets are hypothesized to increase triacylglycerol concentrations by induce fatty acid production and also inhibit the action of lipoprotein lipase (Grundy *et al.,* 2002). Higher triacylglycerol concentrations are related to elevated levels of LDL and lower HDL concentrations, which characterize the dyslipidemia. Apo-$E^{-/-}$ mice have higher LDL levels and the treatments increased that parameter. However animals was received high carbohydrate dietary alone showed reduced triacylglycerol levels compare to others treatments. The liver is a central organ in the regulation of triglyceride

metabolism and accumulation of triglycerides within hepatocytes is the hallmark of fat liver disease (Starley *et al*., 2010, Ferre & Foufelle, 2010). Moreover, this pathology represents a risk factor to cardiovascular diseases (Perazzo *et al*., 2014) and could explain the lowest serum levels of triglycerides in HC group.

The balance between reactive oxygen species (ROS) formation and degradation can be disturbed not only by an increase in its production but also by a decrease in the antioxidant enzyme activities. In supraphisiological doses, the heart is the most frequently affected organ by administration of exogenous steroids (Riezzo *et al*., 2011). It is well established that the heart is most susceptible to free radical damage, due to its intrinsic elevated oxidative metabolic activity and its fragile antioxidant resistance, in comparison to other parts of the body (Vasilaki *et al*., 2016).

Bol alone decreased CAT and SOD activity and enhanced GST activity. The treatments increased IL-6 and IL-2, modulating a pro-inflammatory status. However, significant decreases in the antioxidant enzymes activities might have contributed to increase oxidative stress in mice receiving Bol alone or high carbohydrate dietary only. SOD is considered the first line of defense against deleterious effects of ROS in tissue. In addition, SOD enzyme have been propose to have anti-atherosclerotic effects by inhibition of oxidative alterations (Huige, 2014). Reduction in SOD activity occur because this enzyme can be irreversibly inactivated, until 50% by its product, H_2O_2, in a concentration-dependent (Salo *et al*., 1990). This factor increase atherosclerosis developmental (Amsterdam, 2010). The CAT enzyme is also rapidly inhibited by superoxide anions, final product of enzyme reaction (Kono *et al* 1982), and high concentration from fatty acid levels can modulate catalase activity (Azevedo-Martins *et al*., 2006). In addition, the fact of the heart has lower concentration of CAT, become this organ susceptible to oxidative damage (Hermes-Lima, 2004; Droge, 2002).

Glutathione S-transferases catalyze the conjugation of reduced glutathione to a variety of substrates, including the products of reactive oxygen species (Hayes *et al*., 2005; Welsh *et al*., 2009). The increased activity of GST might be due to a compensatory mechanism in response to increase in the OS

(oxidative stress), generate by administration of boldenoe. It is known that GST activity increase in conditions of oxidative stress (Goncharova *et al*., 2007).

AAS might mediate redox imbalance, regulating antioxidant response generated by modulating signaling pathways. This modulation occur by cytokines and transcription factors of heart tissues (Iwasak *et al*., 2004; Antras-Ferry *et al*., 1997; Pansarasa *et al*., 2002). Boldenone alone was able to modify the oxidative status in heart beyond modulate pro inflammatory immune response. It is now known that all nucleated cell in the myocardium can produce pro inflammatory cytokines in response to injury or OS (Kapadia 1995). The increase of IL-6 and IL-2 concentration promote the inflammation and deterioration of vascular function and heart morphology (Tiyerili *et al*., 2015). High levels of IL-6 are related with heart failure (Plenz, 2001). In addition, the reduction in the levels of IL-10, an anti-inflammatory cytokine, aggravate the tissue damage. Which was confirmed by increase of biomarkers from cardiac damage, CK-MB e LDH.

High carbohydrate intake by Apo-$E^{-/-}$ mice disrupted redox balance and decreased CAT activity, in cardiac tissue. This dietary also increased CK-MB levels, but was unable to change LDH serum levels. However, pro inflammatory cytokines such as IL-10, IL-2, IL-17, INF-γ, and mainly TNF-α, were increased with dietary intake. High carbohydrate intake induce oxidative stress and proinflamatory status. Moreover, the glucose leads to an increase in the reactive oxygen species, generated by mononuclear cells, and inflammation as reflected in an increase in nuclear factor-κβ (Esposito *et al*., 2002; Aljada *et al*., 2004, 2006). Higher concentrations of TNF-α are related to acute myocardial infarction in individuals who present dyslipidemia or atherosclerosis, (Bennet *et al*., 2006) as well as changes in the metabolism of lipids (Sethi *et al*., 1999). IL-2 plays a critical role in the immunological activation of atherosclerotic lesions while IFN-γ can promote vascular remodeling through induction foam cell formation (Ait-Oufella *et al*., 2011), typical characteristic of atherosclerosis. However, when IL-10 is produced in atherosclerotisis occur reduction in the expression of inflammatory mediators (Uyemura *et al*., 1996) lesions. The certain role of IL-17 in atherosclerosis is controversial (Taleb *et al*., 2010). Recent study showed that promoting Th17 responses by genetic inactivation of SOCS3 in T cells reduced atherosclerosis in $LDLr^{-/-}$ mice (Taleb *et al*., 2009)

whereas some data showed that IL-17 has a proatherogenic role. (Erbel *et al*., 2009; Chen *et al*., 2010; Gao *et al*., 2010; Smith *et al*., 2010).

The cardiac tissue damage was confirmed in the present study by rising of injury marker. The excessive production of ROS from auto oxidation of glucose (Wolff 1993 Esposito *et al*., 2002; Aljada *et al*., 2004, 2006), glycated proteins and glycation of antioxidant enzymes, which limit their capacity to detoxify ROS (Giugliano *et al*., 1995), can lead to injury in cardiac tissue. However, increased GST activity and higher IL-10 serum levels, an anti-inflammatory cytokine that has a strong antiatherogenic properties (Yoshioka *et al*., 2004; Hansson *et al*., 2003) induce a cardioprotective effect, preserving morphology of tissue.

The association between boldenone and high carbohydrate dietary intake this not changed first defense line against OS. Besides, the activity of SOD and CAT this not change in the animals was received the higher dose of boldenone. However, the activity of GST increased in both treatments, higher and the lowest dose of boldenone (20mg/kg and 10mg/kg). OS was enable to induce tissue damage in heart, since CK-MB and LDH did not change. The lowest dose did not decreased CAT activity such as Bol and HC treatments, but increase SOD activity. Therefore, the increased of activity SOD and catalase in combination retards the development of atherosclerosis in Apo-$E^{-/-}$ mice (Yang 2004, 2009). Increased GST activity may have cardioprotective effect.

Higher dose of boldenone increased pro inflammatory cytokines IL-2 and INF-γ. While the lowest dose increased levels of IL-6 and IFN-γ. It is not knows the true effects of intake carbohydrate, long term, on inflammatory markers (Santos *et al*., 2012), but high intake of carbohydrate increase proinflammatory cytokines serum levels (Huffman *et al*., 2007; Buyken *et al*., 2010). The increase in serum levels of IFN-γ was seen in all groups who ingested the dietary. IFN-γ is considered a proatherogenic cytokine (Hansson *et al*., 1989; Frostegard *et al*., 1999; Tedgui & Mallat 2006). IL-2 and IFN-γ are associated to Th1 pattern of response, exacerbating atherosclerosis state (Tedgui & Mallat 2006). The intake of large amounts of carbohydrate can increase the oxidative stress.

HC and Bol treatments have not exercised detectable influence on the morphology of the cardiac parenchyma, tissue vascularization was markedly

increased by both treatments, especially when combined and in a dose-dependent way. In addition, the treatment with boldenone might have contributed to increase cardiosomatic index, showing a pathological heart hypertrophy. Vascularization plays a critical role in physiological processes such as repair a damaged tissue. Testosterone improve angiogenisis events in endothelial cells, including migration, proliferation and tubulogenesis (Ng *et al*., 2003, Cai *et al*., 2011). The presence of inflammatory infiltrate with a predominance of mononuclear occur due to a long term treatment, 8 weeks.

5 Conclusion

Taken together, our findings indicated that both HC and Bol can independently aggravated the dyslipidemia, the major risk factor of atherosclerosis, and induce a marked vascular remodeling in cardiac tissue. Apparently, HC is potentially more dangerous that Bol, inducing a marked pro-inflammatory systemic status, which is potentially related to the cardiac inflammatory infiltrate in Apo-$E^{-/-}$ mice. Although HC and Bol can impair the activities of antioxidant enzymes, this effect seem to be not harmful to the cardiac redox balance.

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7 Conflict of Interest

The author(s) declare(s) that they have no conflict of interest to disclose.

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