Universidade Federal do Rio Grande do Sul
Programa de Pós-Graduação em Ciências da Saúde: Cardiologia e Ciências Cardiovasculares

Repercussões cardiovasculares da ingestão aguda de álcool

Tese de Doutorado

Paulo Fernando Dotto Bau

Orientador: Flávio Danni Fuchs

Porto Alegre

2009

Dedicatória

À minha esposa Roseane e aos meus filhos Sophia e Felipe, com amor.

Agradecimentos

Ao meu orientador Prof. Dr. Flávio Danni Fuchs, pela atenção, disponibilidade e competência na condução dessa Tese;

Ao meu irmão Prof. Dr. Claiton H. D. Bau, pela amizade, estímulo constante e orientação;

Aos Prof. Dr. Guido A. Rosito e Prof. Dr. Ruy S. Moraes, pela co-orientação especializada;

Ao Eng. Elton Ferlin, pela competente análise dos dados de Variabilidade da Frequência Cardíaca;

Aos meus pais, sogros (in memoriam), irmãos, cunhados e sobrinhos, pelo apoio incondicional;

Aos colegas e funcionários do ICARDIO, Instituto Cardiovascular, pela amizade e receptividade no uso de sua estrutura para o desenvolvimento do projeto;

Aos colegas do Departamento de Clínica Médica do Centro de Ciências da Saúde da Universidade Federal de Santa Maria, pela liberação e substituição nas atividades docentes;

Às secretárias do consultório, pelo imprescindível apoio diário;

Aos professores, colegas e funcionários do Programa de Pós-Graduação em Ciências da Saúde: Cardiologia e Ciências Cardiovasculares – UFRGS, pelas sugestões e companheirismo.

Ao Vilceu Niederauer, meu educador físico e aos amigos da Academia Fisiocardio, pela ótima estrutura disponibilizada.

A todos os amigos que me incentivaram;

Aos indivíduos voluntários desse estudo, pela inestimável cooperação;

ÍNDICE

Lista de abreviaturas

DAB – diâmetro da artéria braquial

FC – freqüência cardíaca

FMD – vasodilatação mediada pelo fluxo

HDL - lipoproteínas de alta densidade

LDL - lipoproteínas de baixa densidade

MAPA – monitorização ambulatorial da pressão arterial

NFMD – vasodilatação não mediada pelo fluxo

PA – pressão arterial

PAD - pressão arterial diastólica

PAS - pressão arterial sistólica

PNN50 - percentual de diferenças entre intervalos R-R adjacentes maiores de 50 ms

RMSSD - raiz quadrada média das diferenças sucessivas dos intervalos R-R adjacentes

SDNN - desvio padrão dos intervalos R-R

VFC – variabilidade da freqüência cardíaca

Resumo

Introdução: A pressão arterial (PA), a variabilidade da freqüência cardíaca (VFC) e o consumo de bebidas têm sido associados com desfechos cardiovasculares, que têm maior incidência de eventos no período da manhã.

Objetivos: Avaliar em indivíduos jovens e saudáveis a variação em parâmetros cardiovasculares (diâmetro da artéria braquial - DAB, dilatação mediada pelo fluxo - FMD, dilatação não mediada pelo fluxo - NFMD, freqüência cardíaca - FC, PA, VFC) em diferentes momentos do dia. Investigar se a ingestão aguda de álcool tem um efeito em diferentes horários após consumo sobre vários índices da VFC no domínio do tempo, e se tal efeito é independente ou não da FC.

Métodos: Os parâmetros foram mensurados em três horários (17h, 22h e 7h) por ecodoppler da artéria braquial (DAB, FMD, NFMD) e avaliação clínica (PA, FC) em 50 indivíduos saudáveis do sexo masculino, com idades entre 18 e 25 anos (média de 20,74 anos), A VFC foi avaliada por Holter e comparada entre indivíduos jovens e saudáveis que consumiram 60g de álcool ou placebo (35 indivíduos por grupo) durante 17h após a ingestão.

Resultados: O DAB foi menor às 7h quando comparado às 17h e 22h **(**$P < 0.001$). Os valores de FMD não se modificaram ao longo do dia, enquanto o NFMD aumentou às 7h, quando comparado aos demais horários. A VFC caiu após a ingestão de álcool, paralelamente a um aumento na freqüência cardíaca. A variação hora a hora da VFC e FC foram simetricamente opostas, sugerindo que a redução da VFC seria uma conseqüência da ativação simpática associada ao aumento da FC.

Conclusões: O estado fisiológico de vasoconstrição após acordar, com reatividade endotelial e capacidade de dilatação preservada pela manhã, deveria ser

considerado como parte de uma adaptação cardiovascular saudável e levada em conta nos estudos sobre fatores de risco e disfunção endotelial em uma idade mais tardia. Os efeitos autonômicos recorrentes da ingestão de álcool, particularmente em grandes quantidades e por indivíduos com um padrão de consumo abusivo, poderiam estar relacionados com os efeitos deletérios do álcool sobre o coração. Os dois conjuntos de resultados desta Tese apontam para a necessidade de mais estudos controlados em uma faixa etária mais avançada, em indivíduos de ambos os sexos, hipertensos ou normotensos, para avaliar tanto o risco coronariano matinal como também os efeitos do consumo de álcool sobre o aumento da PA e potencial arritmogênico.

Abstract

Introduction: The blood pressure (BP), heart rate variability (HRV) and alcoholic beverage consumption have been associated with negative cardiovascular outcomes which in turn have a higher incidence in the morning period.

Objectives: To evaluate in health young men the variation in cardiovascular parameters (brachial artery diameter - BAD, flow mediated dilation - FMD, non flow mediated dilation - NFMD, heart rate - HR, BP and HRV) in different times of day. To verify if an acute ingestion of alcohol has a time-dependent influence over time domain indices of HRV, and if this effect is independent of heart rate.

Methods: The parameteres were measured in three different times (7 am, 5 pm and 10 pm) by brachial artery ultrasound (BAD, FMD, NFMD) and clinical evaluation (BP, HR) in 50 healthy young males, age range: 18 to 25 years, averaging 20,8 years. HRV was evaluated by Holter and compared between healthy young males who ingested 60 g of ethanol or placebo (35 per group) before and during 17 hours after ingestion.

Results: BAD was smaller at 7 am in comparison with 5 pm and 10 pm ($P < 0.001$). FMD values did not change significantly during the day, while NFMD increased more at 7 am, when compared to the other times of day ($P = 0.04$). There was a fall in HRV after the ingestion of alcohol, accompanied by an increase in heart rate. The hourly change in heart rate and HRV measures were roughly symmetrically opposed to each other, suggesting that the acute ingestion of alcohol promoted a variation in HRV as a consequence of sympathetic activation and increased heart rate.

Conclusions: The physiological state of vasoconstriction after awakening, with preserved capability to dilate in the morning, should be considered to be part of the healthy cardiovascular adaptation before considering later life risk factors and

endothelial dysfunction. The recurrent autonomic effects of alcohol consumption, particularly at higher amounts by individuals with a pattern of abusive consumption, could be related to the deleterious effects of alcohol over the heart. The two sets of results of this Thesis point towards the need for further controlled studies focused on an advanced age range, in subjects of both genders, hipertensives or normotensives, in order to evaluate both the morning coronary risk as well as the effects of alcohol consumption on hypertension and arrythmias.

Capítulo I - Introdução

Introdução

Álcool e doença cardiovascular

Vários estudos sugeriram uma correlação inversa entre consumo moderado de álcool e mortalidade coronariana (Albert e cols., 1999; Rimm e cols., 1999; Belleville e cols., 2002; Grombaek e cols., 2002). No entanto, doses altas de álcool aumentam o risco de mortalidade por várias causas, incluindo cirrose e câncer (Marmot e cols., 1981; Friedman e cols., 1986; Renaud e cols., 1998) Isso resulta em uma curva tipo J entre mortalidade e consumo de álcool (Klatsky e cols., 1992). De acordo com as diretrizes dos departamentos federais de agricultura e saúde dos Estados Unidos *(USDA/ DHHS Dietary Guidelines, 2005)*, com base em um relatório do instituto nacional de abuso de álcool e alcoolismo do mesmo país (Gunzenrath e cols., 2004), o consumo moderado de álcool corresponderia a 1 a 2 doses por dia (15 a 30 g de álcool), que é a quantidade associada com a menor mortalidade por todas as causas.

No início do século XX, observou-se pela primeira vez essa relação inversa entre o consumo de álcool e doença aterosclerótica (Cabot 1904 *apud* Klatsky, 2002). O termo “paradoxo francês” foi cunhado por epidemiologistas para chamar a atenção sobre a incidência relativamente baixa de doença coronariana na população francesa, apesar do grande consumo de gorduras saturadas (Artaud-Wild e cols., 1993; Tunstall-Pedoe e cols., 1999; Belleville, 2002).

O projeto WHO-MONICA (“monitoring trends and determinants in cardiovascular disease”) foi delineado para analisar as relações entre fatores de risco e eventos cardiovasculares. Os dados foram coletados de mais de 100.000 homens e mulheres, de 38 populações, em 21 países (Kuulasmaa e cols., 2000; Tunstall-Pedoe e cols., 2000). Esse estudo confirmou a existência de um gradiente

decrescente na freqüência de eventos coronarianos, do norte para o sul da Europa, com os maiores valores encontrados na Escócia e os menores na Espanha e sudoeste da França. A menor freqüência de eventos coronarianos na França e outros países mediterrâneos foi associada com um escore de risco comparável ao encontrado em populações de outros países desenvolvidos. Entretanto, a razão dessa menor incidência de eventos coronarianos nas populações mediterrâneas ainda não foi explicada. Embora o vinho seja apenas um dos componentes da dieta mediterrânea, foi sugerido que pudesse ter efeito benéfico contra a doença coronariana (Marmot e cols., 1981).

Apesar desses dados favoráveis ao vinho, não há consenso quanto ao tipo de bebida (vinho, cerveja ou destilados), bem como a quantidade que produziria esse impacto favorável das bebidas alcoólicas (Klatsky e cols., 1997; Goldberg e cols., 2001; Di Castelnuovo e cols., 2002). Para determinar os possíveis efeitos benéficos dos componentes alcoólicos e não-alcoólicos (ex: polifenóis) do vinho tinto, um estudo (Senault e cols., 2000) comparou o vinho, uma solução com a mesma quantidade de álcool e vinho tinto sem álcool, verificando um efeito benéfico sobre os níveis lipídicos um pouco maior com o consumo moderado de vinho tinto com álcool, provavelmente devido à ação do álcool e dos polifenóis sobre as lipoproteínas. O efeito antioxidante de vários compostos presentes na casca da uva, como o resveratrol, quercetina e ácido tânico diminuem a oxidação das lipoproteínas de baixa densidade (LDL) e a doença cardiovascular (Belleville, 2002). A diferença fundamental entre os vinhos tinto e branco é o conteúdo fenólico, 20 vezes superior no vinho tinto (Belleville, 2002).

O aparente efeito benéfico do álcool poderia na realidade ser devido a um viés relacionado com o estilo de vida dos usuários de bebidas alcoólicas. Nesse

sentido o “Atherosclerosis Risk in Communities Study” não demonstrou uma direção consistente para as associações entre consumo de álcool e doença coronariana em homens brancos e negros. O efeito seria protetor para os brancos, e prejudicial para os negros. Além disso, o efeito protetor seria mais intenso entre os brancos que consumiam raramente as bebidas alcoólicas. Esses dados sugerem que os níveis de consumo de álcool poderiam ser apenas indicadores indiretos das causas reais de doenças das artérias coronárias (Fuchs e cols., 2004, Fuchs e Chambless, 2007).

Efeitos do álcool sobre a pressão arterial (PA)

O consumo de álcool é um fator de risco estabelecido para a hipertensão. Verificou-se que o consumo diário de álcool aumentaria a pressão arterial (PA) sistólica em aproximadamente 2 mmHg em homens normotensos que consumiam quatro doses diárias de álcool (vinho tinto ou cerveja) (Zilkens e cols., 2005). McFarlane e cols. (2007) compararam os efeitos do álcool sobre a PA em homens e mulheres de uma amostra de base populacional. Eles verificaram que o consumo de mais de duas doses diárias de álcool aumentava a PA em homens, mas não em mulheres.

Tendo em conta os dados epidemiológicos sobre a associação entre o álcool e a PA, foram realizados vários experimentos controlados comparando a PA entre indivíduos que consumiram ou não uma dose de álcool antes do período da avaliação. McFadden e cols., (2005) revisaram nove estudos publicados entre 1984 e 1992, verificando que o álcool provocava um pequeno porém significativo aumento na PA considerando a média das avaliações durante 24 horas. A maioria desses estudos dispunha de números amostrais muito pequenos, com poucas mulheres. Apenas uma avaliação que incluiu hipertensos comparou seus resultados com

normotensos, sugerindo que o aumento da pressão só seria significativo entre os hipertensos (Malhotra e cols., 1985). No entanto, a precisão dessas avaliações pode ter sido prejudicada já que elas precederam o estudo de Abe e cols. (1994), que demonstrou que a resposta pressórica ao álcool não era linear. O efeito do álcool na monitorização ambulatorial da PA de 24 horas (MAPA) revelou um efeito bifásico, com um valor mais baixo 4 h após o consumo, e um pico de elevação após 10 h (Abe e cols., 1994; Rosito e cols., 1999, Bau e cols., 2005), mais pronunciado com uma dose mais alta (Rosito e cols., 1999). Há apenas um estudo comparando a evolução temporal do efeito agudo do álcool sobre a PA em homens e mulheres (Moreira e cols., 1998), com base em uma amostra populacional onde uma das variáveis consideradas foi o momento da última dose de álcool. Os dados sugeriram que o aumento da PA nas mulheres se daria de maneira mais intensa e rápida do que nos homens.

Não há relatos comparando a resposta bifásica ao consumo agudo de álcool em normotensos e hipertensos. A presença de uma elevação mais acentuada da PA em indivíduos hipertensos no período entre 10 e 14 h após o consumo poderia representar um aumento ainda maior no risco cardiovascular, tornando muito questionável a existência de benefícios advindos do consumo de álcool nesses indivíduos.

A redução na PA induzida pelo álcool é considerada como uma resposta da vasodilatação (Abe e cols., 1994), mas até o momento nenhum mecanismo fisiológico foi capaz de explicar convincentemente as respostas hemodinâmicas do álcool (Vlachopoulos e cols., 2003; Fazio e cols., 2004). Várias hipóteses foram levantadas para explicar o aumento na PA algumas horas após o consumo de álcool. Uma delas propõe que o aumento poderia resultar de uma propriedade

intrínseca do etanol de alterar a atividade de canais iônicos das membranas celulares (Tawakol e cols., 2004). Na segunda hipótese, a vasodilatação inicial poderia desencadear um aumento compensatório na atividade simpática (Randin e cols., 1995). Outra possibilidade seria de que a retenção de sódio induzida pelo álcool participaria da elevação subsequente na PA (Kawano e cols., 2004).

Álcool e função endotelial

A camada endotelial da parede vascular possui características endócrinas, produzindo substâncias que agem localmente e a distância, em várias partes do organismo (Furchgott e Zawadzki, 1980; Vapaatalo e Mervaala, 2001). O endotélio é sensível às mudanças de fluxo sangüíneo, pressão arterial, sinais inflamatórios e hormônios circulantes, com capacidade de integrar sinais hemodinâmicos e humorais e modular o tônus vasomotor, de acordo com suas necessidades metabólicas teciduais locais.

A disfunção endotelial precede a formação da placa ateromatosa e tem valor preditivo para o desenvolvimento de doenças cardiovasculares (Schachinger e cols., 2000). A avaliação da vasodilatação mediada pelo fluxo, endotélio dependente (FMD) e da vasodilatação independente do endotélio, mediada pela nitroglicerina (NFMD) com um ultra-som é um método não-invasivo e validado para a medida da função endotelial (Corretti e cols., 2002). Uma resposta endotelial prejudicada está associada com vários fatores de risco para doença cardiovascular (Gaenzer e cols., 2000; Vapaatalo & Mervaala, 2001).

Embora o abuso de álcool tenha sido associado com redução da FMD (Maiorano e cols., 1999; Puddey e cols., 2001), o mesmo não parece ocorrer com bebedores moderados (Zilkens e cols., 2003). Está bem definido que a principal

conseqüência vascular de uma dose aguda de álcool é a vasodilatação (Agewall e cols., 2000; Bau e cols., 2005; Hashimoto e cols., 2001; Vlachopoulos e cols., 2003; Tawakol e cols., 2004); no entanto, não existe consenso quanto à sua ação no FMD. Enquanto Hashimoto e cols. (2001) observaram uma redução no FMD após o uso agudo de álcool, outros não observaram nenhuma alteração (Djousse e cols., 1999; Agewall e cols., 2000). Um estudo do nosso grupo (Bau e cols., 2005) avaliou os efeitos precoces e tardios do álcool sobre a função endotelial em uma amostra homogênea de homens jovens e saudáveis. Verificou-se importante vasodilatação 4h após o consumo, mas nenhum efeito tardio (após 13h) sobre a função endotelial, embora tenha sido observado um efeito bifásico sobre a PA.

Uma limitação importante nos estudos sobre os efeitos do álcool sobre a função endotelial é a escassez de estudos sobre a variação ao longo do dia dos parâmetros fisiológicos relacionados com o diâmetro da artéria braquial e função endotelial, fundamentais para os estudos de fisiologia cardiovascular. Por exemplo, a maior incidência de evento cardiovasculares no período da manhã poderia talvez ser explicada por um menor diâmetro ou menor reatividade vascular pela manhã. Os dois principais estudos que avaliaram tais dados contavam com um tamanho amostral muito pequeno (Etsuda e cols., 1999) ou com uma amostra heterogênea e de faixa etária elevada (Otto e cols., 2004).

Outro tema sob discussão é a dimensão do efeito endotelial atribuível ao álcool em si ou a outros elementos contidos nas bebidas alcoólicas, em especial o vinho tinto. A injúria endotelial pelo LDL oxidado está envolvida na aterogênese e na trombose. Assim, a prevenção da oxidação do LDL pelos polifenóis do vinho ou por outros extratos alcoólicos pode ter um efeito anti-trombótico e anti-aterosclerótico (Ridker e cols., 1994). Um estudo de 15 pacientes com doença arterial coronariana e

prejuízo da função endotelial demonstrou que a ingestão regular, mas por curto espaço de tempo, de suco de uva tinta melhorou significativamente a função endotelial e reduziu a suscetibilidade do LDL à oxidação, sugerindo que os flavonóides e outros componentes possam prevenir eventos cardiovasculares, independentemente do conteúdo alcoólico (Stein e cols., 1999).

Embora possam existir efeitos específicos do vinho, há indícios de um efeito benéfico sobre o endotélio devido especificamente ao etanol, independentemente do tipo de bebida alcoólica (Goldberg e cols., 2001). O embasamento para esse possível efeito protetor do álcool em baixas doses ainda não foi bem estabelecido, mas tem sido atribuído à ação do álcool sobre outros fatores de risco. Em particular, o papel do álcool em aumentar as lipoproteínas de alta densidade (HDL) parece contribuir para uma redução no risco coronariano (Stoclet e cols., 2001). Também se sugeriu que os efeitos do álcool sobre as artérias seriam mediados em parte por uma desaceleração da aterogênese (Kiechl e cols., 1998).

Há evidências de que as vias mediadoras iniciais da ação do álcool sobre o endotélio vascular seriam o estresse oxidativo, lipoproteínas, resistência à insulina e produção de produtos finais da glicosilação avançada (AGEs). A proteção ou dano induzidos pelo álcool sobre esses mecanismos poderiam estar relacionados com a síntese ou ação de vários marcadores, como o óxido nítrico, cortisol, endotelina-1, moléculas de adesão, fator de necrose tumoral alfa, interleucina-6, proteína c-reativa e fatores hemostáticos. A expressão desses marcadores é coerente com a curva J entre o consumo de álcool e saúde cardiovascular. Ou seja, de maneira geral, os níveis dessas substâncias se mostram bastante diferentes quando o consumo de álcool é moderado ou intenso. Na publicação referente ao Capítulo 2 dessa Tese (Bau e cols., 2007) apresentamos uma revisão extensa desses mecanismos.

Efeitos do álcool sobre a variabilidade da frequência cardíaca (VFC)

A redução na VFC está associada a um aumento no risco de eventos cardíacos e mortalidade (Tsuji e cols., 1996a; Lauer, 2009). Além disso, demostrou-se que flutuações na freqüência cardíaca relacionadas com o ritmo circadiano endógeno aumentam a vulnerabilidade cardiovascular pela manhã, com um pico em torno das 10 horas (Hu e cols., 2004). Eventos cardiovasculares tais como morte súbita, acidente vascular cerebral e síndromes coronarianas agudas apresentam um pico de incidência pela manhã (Muller e cols., 1985; Cannon e cols., 1997). Assim, estes dados sugerem que alterações da variabilidade da freqüência cardíaca dentro dos limites da normalidade podem ter grande importância sobre o risco cardiovascular.

Há evidências de que o álcool pode influenciar o sistema nervoso autônomo e a regulação do ritmo cardíaco. A síndrome da arritmia pós-feriado (“holiday heart”), típica dos finais-de-semana, caracteriza-se por fibrilação atrial secundária à ingestão aguda de álcool (Ettinger e cols., 1978; Nissen e Lemberg, 1984; Rich e cols., 1985). Entretando, o mecanismo exato que vincula o consumo de álcool com as arritmias ainda não está completamente estabelecido.

O efeito do álcool sobre a VFC pode ser um dos mecanismos arritmogênicos (Britton e cols., 2008). Vários estudos transversais têm avaliado a relação entre o consumo de álcool e a VFC. Alguns demonstraram que o consumo de álcool diminui a VFC (Masters e cols., 2004; Thayer e cols., 2006), mas isso poderia ser secundário ao aumento da frequência cardíaca (FC) induzido pela substância (Ryan e Howes, 2002). O ajuste para a FC poderia diminuir o efeito do álcool sobre a VFC, mas poderia também mascarar um efeito real e independente do mesmo sobre a

modulação vagal (Koskinen e cols., 1994). Outras investigações não detectaram um efeito significativo do consumo de álcool sobre a VFC (Kageyama e cols., 1997; Fagard e cols., 1999; Tsuji e cols., 1996b e Britton e cols., 2008).

Alguns desses estudos contavam com um número pequeno de consumidores pesados, e o consumo era auto-referido, limitando a confiabilidade dos dados. Portanto, estudos experimentais são necessários para melhor se compreender as relações entre o consumo de álcool e a VFC. Koskinen e cols. (1994) estudaram 12 homens saudáveis que beberam 1g/kg de álcool e verificaram que o consumo agudo diminuía a raiz quadrada média das diferenças sucessivas dos intervalos R-R adjacentes (RMSSD) durante o breve período estudado (3 horas após o consumo), e essa diferença permanecia significativa mesmo quando controlada para a FC. Os autores sugeriram que o efeito residual sobre a VFC poderia ser explicado por uma modulação vagal reduzida causada pelo consumo de álcool. Vaschillo e cols. (2008) compararam parâmetros de VFC em indivíduos que ingeriram álcool, placebo e controles (n=12 em cada grupo). Os dados não foram ajustados para a FC, sugerindo que o álcool reduzia a VFC.

Referências bibliográficas da introdução

Abe H, Kawano Y, Kojima S, Ashida T, Kuramochi M, Matsuoka H, Omae, T. Biphasic effects of repeated alcohol intake on 24-hour blood pressure in hypertensive patients. Circulation. 1994;89:2626-2633.

Agewall S, Wright S, Doughty RN, Whalley GA, Duxbury M, Sharpe N. Does a glass of red wine improve endothelial function? Eur Heart J. 2000;21:74-78.

Albert CM, Manson JE, Cook NR, Ajani UA, Gaziano JM, Hennekens CH. Moderate alcohol consumption and the risk of sudden cardiac death among US male physicians. Circulation. 1999;100:944-950.

Artaud-Wild SM, Connor SL, Sexton G, Connor WE. Differences in coronary mortality can be explained by differences in cholesterol and saturated fat intakes in 40 countries but not in France and Finland. A paradox. Circulation. 1993;88:2771-2779.

Bau PF, Bau CH, Naujorks AA, Rosito GA. Early and late effects of alcohol on blood pressure and endothelial function. Alcohol. 2005;37:53-58.

Bau PF, Bau CH, Rosito GA, Manfroi WC, Fuchs FD. Alcohol consumption, cardiovascular health, and endothelial function markers. Alcohol. 2007;41:479-488.

Belleville J. The French paradox: possible involvement of ethanol in the protective effect against cardiovascular diseases. Nutrition. 2002;18:173-177.

Britton A, Malik M, Marmot M. The cardioprotective effects of alcohol consumption: does cardiac autonomic function play a role? Eur J Epidemiol. 2008;23:105-108.

Cannon CP, McCabe CH, Stone PH, Rogers WJ, Schactman M, Thompson BW, Pearce DJ, Diver DJ, Kells C, Feldman T, Williams M, Gibson RS, Kronenberg MW, Ganz LI, Anderson HV, Braunwald E. The electrocardiogram predicts one-

year outcome of patients with unstable angina and non-Q wave myocardial infarction: results of the TIMI III Registry ECG Ancillary Study. Thrombolysis in Myocardial Ischemia. J Am Coll Cardiol. 1997;30:133-140.

Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. J Am Coll Cardiol. 2002;39:257-265.

Di Castelnuovo A, Rotondo S, Iacoviello L, Donati MB, De Gaetano G. Meta-analysis of wine and beer consumption in relation to vascular risk. Circulation. 2002;105:2836-2844.

Djousse L, Ellison RC, McLennan CE, Cupples LA, Lipinska I, Tofler GH, Gokce, N Vita JA. Acute effects of a high-fat meal with and without red wine on endothelial function in healthy subjects. Am J Cardiol. 1999;84:660-664.

Etsuda H, Takase B, Uehata A, Kusano H, Hamabe A, Kuhara R, Akima T, Matsushima Y, Arakawa K, Satomura K, Kurita A, Ohsuzu F. Morning attenuation of endothelium-dependent, flow-mediated dilation in healthy young men: possible connection to morning peak of cardiac events? Clin Cardiol. 1999;22:417-421.

Ettinger PO, Wu CF, De La Cruz C Jr, Weisse AB, Ahmed SS, Regan TJ. Arrhythmias and the "Holiday Heart": alcohol-associated cardiac rhythm disorders. Am Heart J. 1978;95:555-562.

Fagard RH, Pardaens K, Staessen JA. Influence of demographic, anthropometric and lifestyle characteristics on heart rate and its variability in the population. J Hypertens. 1999;17:1589-1599.

Fazio M, Bardelli M, Fabris B, Macaluso L, Fiammengo F, Vran F, Bossi M, Candido R, Gerloni R, Carretta R. Large-artery hemodynamics after acute alcohol administration in young, healthy volunteers. Angiology. 2004;55:139-145.

Friedman LA, Kimball AW. Coronary heart disease mortality and alcohol consumption in Framingham. Am J Epidemiol. 1986;124:481-489.

Fuchs FD, Chambless LE, Folsom AR, Eigenbrodt ML, Duncan BB, Gilbert A, Szklo M. Association between alcoholic beverage consumption and incidence of coronary heart disease in whites and blacks: the Atherosclerosis Risk in Communities Study. Am. J. Epidemiol. 2004;160: 466-474.

Fuchs FD, Chambless LE. Is the cardioprotective effect of alcohol real? Alcohol. 2007;41:399-402.

Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature. 1980;288:373-376.

Gaenzer H, Sturm W, Kirchmair R, Neumayr G, Ritsch A, Patsch J. Circadian variation of endothelium-dependent vasodilatation of the brachial artery as a confounding factor in the evaluation of endothelial function. Atherosclerosis. 2000;149:227-228.

Goldberg IJ, Mosca L, Piano MR, Fisher EA. Wine and your heart. Circulation. 2001;103:472-475.

Gronbaek M. Alcohol, type of alcohol, and all-cause and coronary heart disease mortality. Ann N Y Acad Sci. 2002;957:16-20.

Gunzerath, L., Faden, V., Zakhari, S., Warren, K. National Institute on Alcohol Abuse and Alcoholism report on moderate drinking. Alcohol. Clin. Exp. Res. 2004;28:829-847.

Haak T, Marz W, Jungmann E, Hausser S, Siekmeier R, Gross W, Usadel KH. Elevated endothelin levels in patients with hyperlipoproteinemia. Clin Investig. 1994;72:580-584.

Hashimoto M, Kim S, Eto M, Iijima K, Ako J, Yoshizumi M, Akishita M, Kondo K, Itakura H, Hosoda K, Toba K, Ouchi Y. Effect of acute intake of red wine on flow-mediated vasodilatation of the brachial artery. Am J Cardiol. 2001;88:1457-1460.

Hu K, Ivanov PCh, Hilton MF, Chen Z, Ayers RT, Stanley HE, Shea SA. Endogenous circadian rhythm in an index of cardiac vulnerability independent of changes in behavior. Proc Natl Acad Sci U S A. 2004;101:18223-18227.

Kageyama T, Nishikido N, Honda Y, Kurokawa Y, Imai H, Kobayashi T, Kaneko T, Kabuto M. Effects of obesity, current smoking status, and alcohol consumption on heart rate variability in male white-collar workers. Int Arch Occup Environ Health. 1997;69:447-454.

Kawano Y, Abe H, Kojima S, Takishita S, Matsuoka H. Effects of repeated alcohol intake on blood pressure and sodium balance in Japanese males with hypertension. Hypertens Res. 2004;27:167-172.

Kiechl S, Willeit J, Rungger G, Egger G, Oberhollenzer F, Bonora E. Alcohol consumption and atherosclerosis: what is the relation? Prospective results from the Bruneck Study. Stroke. 1998;29:900-907.

Klatsky AL, Armstrong MA, Friedman GD. Alcohol and mortality. Ann Intern Med. 1992;117:646-654.

Klatsky AL, Armstrong MA, Friedman GD. Red wine, white wine, liquor, beer, and risk for coronary artery disease hospitalization. Am J Cardiol. 1997;80:416-420.

Klatsky AL. Alcohol and cardiovascular diseases: a historical overview. Ann N Y Acad Sci. 2002;957:7-15.

Koskinen P, Virolainen J, Kupari M. Acute alcohol intake decreases short-term heart rate variability in healthy subjects. Clin Sci (Lond). 1994;87:225-230.

Kuulasmaa K, Tunstall-Pedoe H, Dobson A, Fortmann S, Sans S, Tolonen H, Evans A, Ferrario M, Tuomilehto J. Estimation of contribution of changes in classic risk factors to trends in coronary-event rates across the WHO MONICA Project populations. Lancet. 2000;355:675-687.

Lauer MS. Autonomic function and prognosis. Cleve Clin J Med. 2009;76 Suppl 2:S18-22.

Maiorano G, Bartolomucci F, Contursi V, Minenna FS, Di Mise R, Palasciano A, Allegrini B, Amoruso M, Kozakova M. Noninvasive detection of vascular dysfunction in alcoholic patients. Am J Hypertens. 1999;12:137-144.

Malhotra H, Mehta SR, Mathur D, Khandelwal PD. Pressor effects of alcohol in normotensive and hypertensive subjects. Lancet. 1985;326:584-586.

Marmot MG, Rose G, Shipley MJ, Thomas BJ. Alcohol and mortality: a U-shaped curve. Lancet. 1981;317:580-583.

Masters JA, Stevenson JS, Schaal SF. The association between moderate drinking and heart rate variability in healthy community-dwelling older women. Biol Res Nurs. 2004;5:222-233.

McFadden CB, Brensinger CM, Berlin JA, Townsend RR. Systematic review of the effect of daily alcohol intake on blood pressure. Am J Hypertens. 2005;18:276-286.

McFarlane SI, von Gizycki H, Salifu M, Deshmukh M, Manieram M, Gebreegziabher Y, Gliwa AA, Bordia S, Shah S, Sowers JR. Alcohol consumption and blood pressure in the adult US population: assessment of gender-related effects. J Hypertens. 2007;25:965-970.

Moreira LB, Fuchs FD, Moraes RS, Bredemeier M, Duncan BB. Alcohol intake and blood pressure: the importance of time elapsed since last drink. J Hypertens. 1998;16:175-180.

Muller JE, Stone PH, Turi ZG, Rutherford JD, Czeisler CA, Parker C, Poole WK, Passamani E, Roberts R, Robertson T, et al. Circadian variation in the frequency of onset of acute myocardial infarction. N Engl J Med. 1985;313:1315-1322.

Nissen MB, Lemberg L. The "holiday heart" syndrome. Heart Lung. 1984;13:89-92.

Otto ME, Svatikova A, Barretto RB, Santos S, Hoffmann M, Khandheria B, Somers V. Early morning attenuation of endothelial function in healthy humans. Circulation. 2004;109:2507-2510.

Puddey IB, Zilkens RR, Croft KD, Beilin LJ. Alcohol and endothelial function: a brief review. Clin Exp Pharmacol Physiol. 2001;28:1020-1024.

Randin D, Vollenweider P, Tappy L, Jequier E, Nicod P, Scherrer U. Suppression of alcohol-induced hypertension by dexamethasone. N Engl J Med. 1995;332:1733-1737.

Renaud SC, Gueguen R, Schenker J, d'Houtaud A. Alcohol and mortality in middle-aged men from eastern France. Epidemiology. 1998;9:184-188.

Rich EC, Siebold C, Campion B. Alcohol-related acute atrial fibrillation. A case-control study and review of 40 patients. Arch Intern Med. 1985;145:830-833.

Ridker PM, Vaughan DE, Stampfer MJ, Glynn RJ, Hennekens CH. Association of moderate alcohol consumption and plasma concentration of endogenous tissue-type plasminogen activator. JAMA. 1994;272:929-933.

Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. BMJ. 1999;319:1523-1528.

Rosito GA, Fuchs FD, Duncan BB. Dose-dependent biphasic effect of ethanol on 24-h blood pressure in normotensive subjects. Am J Hypertens. 1999;12:236-240.

Ryan JM, Howes LG. Relations between alcohol consumption, heart rate, and heart rate variability in men. Heart. 2002;88:641-642.

Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation. 2000;101:1899-1906.

Senault C, Betoulle D, Luc G, Hauw P, Rigaud D, Fumeron F. Beneficial effects of a moderate consumption of red wine on cellular cholesterol efflux in young men. Nutr Metab Cardiovasc Dis. 2000;10:63-69.

Stein JH, Keevil JG, Wiebe DA, Aeschlimann S, Folts JD. Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. Circulation. 1999;100:1050-1055.

Stoclet JC. Bonum vinum laetificat cor hominum. Med Sci Monit. 2001;7:842-847.

Tawakol A, Omland T, Creager MA. Direct effect of ethanol on human vascular function. Am J Physiol Heart Circ Physiol. 2004; 286:2468-2473.

Thayer JF, Hall M, Sollers JJ 3rd, Fischer JE. Alcohol use, urinary cortisol, and heart rate variability in apparently healthy men: Evidence for impaired inhibitory control of the HPA axis in heavy drinkers. Int J Psychophysiol. 2006;59:244-250.

Tsuji H, Larson MG, Venditti FJ Jr, Manders ES, Evans JC, Feldman CL, Levy D. Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. Circulation. 1996a;94:2850-2855.

Tsuji H, Venditti FJ Jr, Manders ES, Evans JC, Larson MG, Feldman CL, Levy D. Determinants of heart rate variability. J Am Coll Cardiol. 1996b;28:1539-1546.

Tunstall-Pedoe H, Kuulasmaa K, Mahonen M, Tolonen H, Ruokokoski E, Amouyel P. Contribution of trends in survival and coronary-event rates to changes in coronary heart disease mortality: 10-year results from 37 WHO MONICA project populations. Monitoring trends and determinants in cardiovascular disease. Lancet. 1999;353:1547-1557.

Tunstall-Pedoe H, Vanuzzo D, Hobbs M, Mahonen M, Cepaitis Z, Kuulasmaa K, Keil U. Estimation of contribution of changes in coronary care to improving survival, event rates, and coronary heart disease mortality across the WHO MONICA Project populations. Lancet. 2000;355:688-700.

U.S. Department of Health and Human Services and U.S. Department of Agriculture. (2005). Dietary Guidelines for Americans, 6th Edn (Washington, DC: U.S. Government Printing Office).

Vapaatalo H, Mervaala E. Clinically important factors influencing endothelial function. Med Sci Monit. 2001;7:1075-1085.

Vaschillo EG, Bates ME, Vaschillo B, Lehrer P, Udo T, Mun EY, Ray S. Heart rate variability response to alcohol, placebo, and emotional picture cue challenges: effects of 0.1-Hz stimulation. Psychophysiology. 2008;45:847-858.

Vlachopoulos C, Tsekoura D, Tsiamis E, Panagiotakos D, Stefanadis C. Effect of alcohol on endothelial function in healthy subjects. Vasc Med 2003;8:263-265.

Zilkens RR, Burke V, Hodgson JM, Barden A, Beilin LJ, Puddey IB. Red wine and beer elevate blood pressure in normotensive men. Hypertension 2005;45:874-879.

Zilkens RR, Rich L, Burke V, Beilin LJ, Watts GF, Puddey IB. Effects of alcohol intake on endothelial function in men: a randomized controlled trial. J Hypertens 2003;21:97-103.

Capítulo II

Artigo de Revisão: Alcohol consumption, cardiovascular health, and endothelial function markers

ELSEVIER

Alcohol 41 (2007) 479–488

ALCOHOL

Alcohol consumption, cardiovascular health, and endothelial function markers

Paulo F.D. Bau[a,b,*], Claiton H.D. Bau[c], Guido A. Rosito[d], Waldomiro C. Manfroi[e], Flávio D. Fuchs[e]

[a]Department of Clinical Medicine, Health Sciences Centre, Universidade Federal de Santa Maria, Roraima Avenue 1000, Santa Maria, RS 97105-900, Brazil
[b]Cardiovascular Institute (ICARDIO), Santa Maria, RS, Brazil
[c]Department of Genetics, Institute of Biosciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[d]Department of Public Health, FFFCMPA, Porto Alegre, RS, Brazil
[e]Division of Cardiology, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil

Received 7 June 2007; received in revised form 23 August 2007; accepted 23 August 2007

Abstract

Cardiovascular diseases are among the worldwide leading causes of shorter life expectancy and loss of quality of life. Thus, any influence of diet or life habits on the cardiovascular system may have important implications for public health. Most world populations consume alcoholic beverages. Since alcohol may have both protective and harmful effects on cardiovascular health, the identification of biochemical mechanisms that could explain such paradoxical effects is warranted. The vascular endothelium is the target of important mediating pathways of differential ethanol concentrations, such as oxidative stress, lipoproteins, and insulin resistance. Alcohol-induced endothelial damage or protection may be related to the synthesis or action of several markers, such as nitric oxide, cortisol, endothelin-1, adhesion molecules, tumor necrosis factor alpha, interleukin-6, C-reactive protein, and haemostatic factors. The expression of these markers is consistent with the J-shaped curve between alcohol consumption and cardiovascular health. However, there is genetic and phenotypic heterogeneity in alcohol response, and despite the apparent beneficial biochemical effects of low doses of ethanol, there is not enough clinical and epidemiological evidence to allow the recommendation to consume alcoholic beverages for abstemious individuals. Considering the potential for addiction of alcoholic beverage consumption and other negative consequences of alcohol, it would be worthwhile to identify substances able to mimic the beneficial effects of low doses of ethanol without its adverse effects.

Keywords: Endothelium; Alcohol; Alcoholic beverages; Biochemical markers; Cardiovascular risk; Cardiovascular protection

Alcohol and cardiovascular disease

The inverse correlation between moderate alcohol consumption and coronary mortality has long been recognized (Albert et al., 1999; Belleville, 2002; Klatsky, 2002; Rimm et al., 1999). The term "French paradox" was coined by epidemiologists to call attention to the relatively low rate of coronary disease among the French, despite the heavy consumption of saturated fats (Artaud-Wild et al., 1993; Belleville, 2002; Tunstall-Pedoe et al., 1999). However, high doses of alcohol increase the risk of mortality due to several causes, including cirrhosis and cancer (Friedman and Kimball, 1986; Marmot et al., 1981; Renaud et al., 1998). This results in a J-type curve between mortality and alcohol consumption (Klatsky et al., 1992). Beneficial effects of the consumption of low to moderate amounts of ethanol have been postulated. According to the U.S. Department of Agriculture/U.S. Department of Health and Human Services Dietary Guidelines (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2005), based on a report of U.S. National Institute on Alcohol Abuse and Alcoholism (Gunzerath et al., 2004), moderate drinking of ethanol corresponds to 1–2 drinks per day (15–30 g of alcohol), which is the amount associated with the lowest all cause mortality.

The relationship between alcohol consumption and blood pressure is complex, reproducing the relationship between drinking and cardiovascular disease in general. Heavy alcohol consumption is an established risk factor for hypertension. A study showed that daily alcohol consumption increases systolic blood pressure by approximately 2 mmHg, in normotensive men who take four doses of alcohol, either as

* Corresponding author. Rue Appel, 655/903, 9715-030 Santa Maria, RS, Brazil. Tel.: +55-55-3222-19-29; fax: +55-55 3025-12-12.
E-mail address: paulobau@gmail.com (P.F.D. Bau).

0741-8329/07/$ – see front matter
doi: 10.1016/j.alcohol.2007.08.004

red wine or beer (Zilkens et al., 2005). Commenting on this study, Fuchs (2005) argues that the vasopressor effect of chronic alcohol use observed even at moderate doses, calls for caution in interpreting the studies that indicate reduction of alcohol-related cardiovascular risk.

Paradoxically, alcohol consumption appears to reduce the risk of myocardial infarction among hypertensive patients (Beulens et al., 2007). The response of blood pressure to alcohol occurs in two phases. While in the first hours after consumption there is arterial dilation accompanied by hypotension, approximately 11–13 h after consumption a higher than baseline blood pressure may be detected (Abe et al., 1994; Bau et al., 2005; Rosito et al., 1999).

Data from the WHO-MONICA project (Kuulasmaa et al., 2000; Tunstall-Pedoe et al., 2000) demonstrated a decreasing gradient in the frequency of coronary events, from north to south in Europe. The highest frequency was found in Scotland and the lowest in Spain and Southeast France. The lower frequency of coronary events in France and other Mediterranean countries was associated with a risk score comparable with that found in populations from other developed countries. However, it is not yet known why there is a lower rate of coronary events in the Mediterranean populations. Although wine is only one of the components of the diet in this population, it was suggested that it might have a beneficial effect against coronary disease (Marmot et al., 1981).

Despite these favorable effects of wine, there is no consensus as to the type of beverage (wine, beer, or liquors), as well as the amount that produces the putative benefit of alcoholic beverages (Di Castelnuovo et al., 2002; Goldberg et al., 2001; Klatsky et al., 1997). To determine the possible beneficial effects of the alcoholic and nonalcoholic components of red wine (e.g., polyphenols), a study compared wine, a solution with the same amount of alcohol, and red wine without alcohol (Senault et al., 2000). There was a slightly higher beneficial effect on the lipid levels when there was moderate consumption of red wine with alcohol, probably due to the action of alcohol and polyphenols on the lipoproteins. The antioxidant effect of several compounds present in grape skin, such as resveratrol, quercetine, and tannic acid, decrease low-density lipoprotein (LDL) oxidation and cardiovascular disease. The main difference between red and white wines is the phenolic content, 20 times higher in red wine (Belleville, 2002). However, although there may be specific effects of wine, there are signs of reduction in the risk of coronary disease due specifically to ethanol, independent of type of alcoholic beverage (Goldberg et al., 2001).

Beulens et al. (2007) suggested a hypothesis to explain both the data focusing on red wine and those that suggest that alcohol consumption in itself would have a beneficial effect. According to the authors, it is possible that the most consumed beverage in a given population is the one that is most probably involved in the reduction of cardiovascular disease risk in that sample. Thus, they propose that the inverse association could most likely be due to the effect of alcohol.

Besides the multifactorial nature of the effects of alcohol in the cardiovascular system, a large amount of evidence points toward beneficial and harmful effects of alcohol consumption particularly in endothelial function. The resulting endothelial effects are then related to the levels of several markers that reflect the J-shaped curve.

Endothelial function: general aspects and clinical evaluation

The endothelial layer of the vascular wall has endocrine characteristics, producing substances that act locally and remotely in several parts of the organism (Furchgott and Zawadzki, 1980; Vapaatalo and Mervaala, 2001). The endothelium is sensitive to changes in blood flow, blood pressure, inflammatory signs, and circulating hormones, with a capacity to integrate hemodynamic and humoral signs and to modulate the vasomotor tone according to the local tissue metabolic needs.

Endothelial dysfunction precedes the formation of atheromatous plaque and has a predictive value for the development of cardiovascular diseases (Schachinger et al., 2000). The evaluation of flow-mediated, endothelium dependent dilation (FMD) and endothelium independent, nitroglycerin-mediated dilation (NFMD) by ultrasound is a noninvasive validated method for measuring endothelial function (Corretti et al., 2002). An impaired endothelial response is associated with risk factors for cardiovascular disease (Vapaatalo and Mervaala, 2001).

Although alcohol abuse has been associated with reduction in FMD (Maiorano et al., 1999; Puddey et al., 2001), this does not appear to occur with moderate drinkers (Zilkens et al., 2003). The main vascular consequence of an acute dose of alcohol is vasodilation (Agewall et al., 2000; Bau et al., 2005; Hashimoto et al., 2001), but there is no consensus as to its action in FMD. While one study (Hashimoto et al., 2001) observed a reduction in FMD after acute alcohol use, others did not find any change (Agewall et al., 2000; Djousse et al., 1999). We have evaluated the early and late effects of alcohol on endothelial function in a homogeneous sample of healthy young men (Bau et al., 2005). A significant vasodilation occurred 4 h after consuming 60 g of alcohol, but there was no late effect (after 13 h) on endothelial function, although a biphasic effect on blood pressure was found, with elevation of blood pressure levels during the final period of observation.

Endothelial function: biochemical markers and the influence of alcohol

The vasodilator effect of alcohol is related to a higher expression of the endothelial nitric oxide synthase (eNOS) (Venkov et al., 1999). However, the effect of alcohol on the

endothelial function itself is more complex. The J-shaped relationship with alcohol consumption reported for cardiovascular risk was also observed for the relationship between alcohol consumption and several inflammatory biomarkers (Thorand et al., 2006).

Whereas moderate consumption is supposed to have a risk-reducing effect reflected on these mediators, alcoholism is associated with endothelial dysfunction and a number of unfavorable outcomes, even among apparently healthy former alcoholics (Di Gennaro et al., 2007). Vasdev et al. (2006) reviewed possible biochemical mechanisms for the beneficial effect of low alcohol doses. The authors suggested that the biochemical pathway for the differential effect of low as opposed to high doses would be related to the ability of low alcohol doses to increase the antioxidant activity, lower insulin resistance, and reduce advanced glycation end products (AGEs), therefore preventing hypertension and atherosclerosis. High doses of alcohol, in turn, would have opposite consequences. Table 1 summarizes the effects of alcohol over several biomarkers of endothelial function.

Oxidative stress

In high concentrations, ethanol is initially metabolized by the microsomal ethanol oxidizing system, creating an oxidative environment. In low concentrations, alcohol is first metabolized by alcohol dehydrogenase, producing reduced nicotinamide adenine dinucleotide from NAD, increasing antioxidant capacity (Vasdev et al., 2006). Other alcohol-induced oxidative stress would result from the production of reactive oxygen species by activation of the mitochondrial electron transport chain, enzymes of the cytochrome P450 complex, and phagocytes (Albano, 2006).

Reactive oxygen species are major initial elements by which high alcohol concentration exerts an inflammatory action on the cardiovascular system (Wu et al., 2006). Oxidative stress also appears to be the initial factor in the pathogenesis of alcoholic cardiomyopathy. In this case, alcohol appears to act on lipid peroxidation, induce oxidative damage in the mitochondrial DNA, and reduce the antioxidant defenses in the heart (Chicco et al., 2006). Physical exercise attenuates the prooxidant activity of alcohol in the myocardium, probably because exercise stimulates the antioxidant defense system (Chicco et al., 2006).

It is unclear how oxidative stress acts on a whole range of inflammatory process markers. An investigation examined the association between gamma glutamyl transferase, an oxidative stress marker, and the C-reactive protein (CRP) levels in a sample of 12,110 adults (Lee and Jacobs, 2005). There was a positive correlation between the two markers, suggesting that oxidative stress could be a key component of several subsequent reactions associated with chronic inflammation.

The oxidation of LDL plays an important role in the development of atherosclerosis, and oxidized LDL could have a major role in abnormal endothelial relaxation (Steinberg, 1991). Heavy alcohol consumption could be responsible for increased LDL oxidation, since ethanol in high doses is prooxidant (Puddey et al., 2001; Vasdev et al., 2006).

High-density lipoprotein

There is evidence that a significant part of the association observed between alcohol consumption and coronary disease is mediated by an increase in high-density lipoprotein (HDL) cholesterol (Beulens et al., 2007; Mukamal et al., 2005; Rimm et al., 1999). Schafer et al. (2007) evaluated the influence of nonaddictive alcohol consumption on the composition of HDL and its subfractions. Apart from the expected increase in HDL, they also observed

Table 1
The effect of alcohol on biomarkers of endothelial function: a summary of the main vascular effects

	Effect of alcohol consumption			
Mediator	Moderate	Higher	Adverse vascular effects	Selected references
Oxidative stress	Antioxidant	Oxidant	Proinflammatory	Vasdev et al. (2006); Wu et al. (2006)
HDL	Increase	Increase	Unknown	Schafer et al. (2007)
Insulin resistance	Decrease	Increase	Inconclusive	Bell et al. (2000)
Nitric oxide	Increase	Formation of peroxynitrite	Cytotoxicity	Pacher et al. (2007); Puddey et al. (2001)
Cortisol	Blunted response	Blunted response	Unknown	Dai et al. (2007)
Endothelin	Increase	Increase	Increase blood pressure	Soardo et al. (2006, 2005)
Adhesion molecules	Decrease	Increase	Proinflammatory	Sacanella and Estruch (2003)
TNF-alpha	Decrease	Increase	Endothelial dysfunction	Badia et al. (2004); Luedemann et al. (2005)
IL-6	Decrease	Increase	Proinflammatory	Pai et al. (2006)
CRP	Decrease	Increase	Proinflammatory	Raum et al. (2006); Zhong et al. (2006)
Hemostatic factors	Anticoagulant	Procoagulant	Thrombosis	Lee and Lip (2003); Salem and Laposata (2005)

HDL, high-density lipoproteins; TNF-alpha, tumor necrosis factor alpha; IL-6, interleukin-6; CRP, C-reactive protein.

qualitative changes in HDL. Alcohol increased the lipid content of HDL, augmenting its antiatherogenic effect. In addition, the alcohol-induced phospholipid enrichment of HDL might reduce the inflammatory response of atherogenesis.

HDL has a wide range of properties in the endothelium and is involved in most of the alcohol-related physiological pathways included in this review. HDL is an effective antioxidant, inhibiting the oxidative modification of LDL, reducing its atherogenicity (Barter et al., 2004), and promotes the production of nitric oxide by upregulating eNOS expression (Mineo et al., 2006). The antithrombotic properties of HDL are related to the abilities to attenuate the expression of tissue factor and selectins, to downregulate thrombin generation via the protein C pathway, and to blunt platelet activation (Mineo et al., 2006).

Insulin resistance

Insulin resistance is characterized by an inadequate glucose uptake in peripheral tissues at a given concentration of plasma insulin. It involves an impairment of the nonoxidative (glycolytic) pathways of intracellular glucose metabolism (Ferrannini et al., 1987). As a consequence, there is an increased formation of AGEs, which play a major causative role in hypertension and atherosclerosis through an increase in endothelial dysfunction, inflammatory responses, and increased oxidative stress (Vasdev et al., 2006).

Moderate alcohol consumption has been associated with improved insulin sensitivity in humans (Bell et al., 2000; Lazarus et al., 1997). An analysis of the effect of the intake of various ethanol concentrations in rats showed an inverted U-shaped relationship between alcohol intake and insulin sensitivity (Furuya et al., 2003). Vasdev et al. (2006) hypothesized that by improving insulin resistance, moderate alcohol consumption would limit formation of AGEs and their subsequent hypertensive and atherosclerotic complications. Low ethanol intake may also work to improve insulin resistance by lowering plasma-free fatty acids (Avogaro et al., 2002).

Nitric oxide

Since the classic studies of Furchgott and Zawadzki (1980), it is known that the endothelium produces a substance that is responsible for vasodilation. Later, this substance was characterized as being nitric oxide (Ignarro et al., 1987; Palmer et al., 1987). Nitric oxide and its metabolites have been implicated as clinical markers of endothelial dysfunction (Raitakari and Celermajer, 2000; Vallance and Chan, 2001). Actually, most of the cytotoxicity attributed to nitric oxide is due to peroxynitrite, produced from the reaction between nitric oxide and superoxide (Pacher et al., 2007). Peroxynitrite is implicated in the pathogenesis of cardiovascular disease (Pacher et al., 2005) and complications of diabetes, including the development and progression of diabetic cardiomiopathy, retinopathy, neuropathy, and nephropathy (Pacher and Szabó, 2006). McKim et al. (2003) showed that the production of peroxynitrite is a mediating factor in alcohol liver injury.

The beneficial effect of low alcohol doses on the cardiovascular system has also been related to the release of nitric oxide (Puddey et al., 2001). Abou-Agag et al. (2005) supported this hypothesis when they found that moderate doses of ethanol increased the expression of eNOS in rats. They suggested that the vascular relaxation resulting from an increase in nitric oxide might explain, at least in part, the cardioprotective benefits of moderate alcohol consumption.

Cortisol

Cortisol is involved in responses to stress and other physiological functions. Mental stress, on the other hand, causes damage to the endothelial function that, chronically, may result in speeding up the atherogenic process (Ghiadoni et al., 2000). It is possible that the association between stress and cardiovascular disease is mediated partly by the relationship between environmental stress and serum cortisol levels (Steptoe et al., 2003). Broadley et al. (2005) investigated whether cortisol is a mediating factor of the effect of stress on endothelial function (evaluated by FMD), and the reduction of baroreceptor reflex sensitivity. For this purpose, the authors submitted two groups of individuals to stress, one of them previously treated with a cortisol synthesis inhibitor (metyrapone) and the other receiving placebo. They found that metyrapone reduced cortisol levels and prevented endothelial dysfunction and reduction of the baroreceptor reflex sensitivity. Sillaber and Henniger (2004) suggested that individuals with dysfunctional responses to stress related to the hypothalamo-hypophyseal-adrenocortical axis could present a greater tendency to alcohol consumption as a stress relief factor. This could be related to the fact that alcohol prevents the stress-induced increases in plasma ACTH and cortisol (Dai et al., 2007). Thus, it is possible that some of the mechanisms of alcohol action on endothelial and vascular function could be mediated by cortisol.

Endothelin

Endothelin, a polypeptide with 21 aminoacids, is one of the most potent endogenous vasoconstrictors ever identified (Levin, 1995; Yanagisawa et al., 1988), producing constriction both in veins and in arteries. Several studies on animal models and humans showed the correlation between endothelin production and activity, and some of the main risk factors for atherosclerosis (Haak et al., 1994). A selective antagonist of one of the endothelin receptors (the

endothelin-A receptor) was able to prevent stress-provoked endothelial dysfunction (Spieker et al., 2002).

The hypothalamo-pituitary axis and endothelin interact in their effects on the vasculature (Broadley et al., 2005). Thus, the activation of the vascular endothelin system appears to depend on cortisol (Broadley et al., 2005) and on other cardiovascular stress markers such as norepinephrine (Touyz and Schiffrin, 2003).

Alcohol consumption raises the endothelin levels in a dose-dependent manner, mediated by increased alcohol-induced oxidative stress (Soardo et al., 2005). The authors showed that blocking oxidative stress prevented the functional changes induced by alcohol in the endothelium. In a subsequent study, the same group of researchers showed that the increase in the endothelin levels could be in the pathway of blood pressure elevation induced by heavy alcohol consumption. This hypertension was rapidly reverted when alcohol consumption ceases (Soardo et al., 2006). Zilkens et al. (2005) also included increased endothelin as a mediator of the effect of alcohol on increased blood pressure.

Adhesion molecules

Another indicator of endothelial dysfunction and an important step in atherogenesis is the expression of the adhesion molecules on the surface of the vascular endothelium in response to lesions (Fries et al., 1993). The intercellular adhesion molecule (ICAM-1) enables the adhesion and transmigration of inflammatory cells to the vascular wall (Ridker et al., 1998). The emission of these molecules in plasma can be considered a marker of atherogenesis and endothelial dysfunction. In patients with angina, but free of flow-limiting lesions, elevated levels of this marker can indicate endothelial dysfunction (Clausell et al., 1999). The dose-dependent elevation of ICAM-1 and selectin-E in alcoholics may reflect the direct or indirect effect of alcohol on the endothelial cell (Sacanella et al., 1999). However, moderate alcohol consumption appears to reduce the expression of these molecules in the endothelium (Sacanella and Estruch, 2003). This information was confirmed in a prospective study when alcohol consumption was shown to be inversely associated with the levels of adhesion molecules (Shai et al., 2006).

Tumor necrosis factor alpha

Badia et al. (2004) analyzed the effect of the moderate consumption of red wine or gin on human monocyte adhesion to endothelial cells in healthy men. Tumor necrosis factor alpha (TNF-alpha)–induced adhesion of monocytes to endothelial cells was virtually abolished after red wine consumption but was only partially reduced after gin consumption. The authors suggested that this effect might be due to the downregulation of adhesion molecules on the monocyte surface.

On the other hand, chronic ethanol-induced damage to various organs has been linked to the increased release of TNF-alpha. In vitro studies demonstrated that the endothelial cell proliferation and re-endothelialization is inhibited by TNF-alpha at the sites of arterial injury (Kishore et al., 2003). Luedemann et al. (2005) showed that the presence of ethanol enhances the TNF-alpha–induced endothelial cell dysfunction. The authors hypothesized that chronic ethanol consumption may negatively influence post angioplasty re-endothelialization, thereby contributing to the development of restenosis.

Interleukin-6

Interleukin-6 (IL-6) may also be among the links connecting alcohol consumption and atherosclerosis (Carty, 1999). It is the main interleukin in the acute inflammatory phase (Baumann and Gauldie, 1994). IL-6 regulates genes that encode most of the proteins involved in this stage (Castell et al., 1990), besides suppressing the hepatic synthesis of albumin (Carty, 1999).

In liver disease caused by alcohol there are elevated concentrations of IL-6 and other interleukins (McClain et al., 1999), while moderate alcohol consumption can inhibit IL-6 synthesis or its actions in hepatocytes (Carty, 1999). In this sense, a prospective study showed an inverse relationship between IL-6 and other inflammatory markers and moderate alcohol consumption (Pai et al., 2006). The authors suggested that these inflammatory markers could constitute the mediating factors of the protective action of moderate alcohol consumption on cardiovascular risk. Maraldi et al. (2006), however, could not confirm this hypothesis. They observed that the alcohol-related decrease in cardiovascular risk was independent of IL-6.

CRP

CRP is a marker of the acute-phase response of the inflammatory process produced by the liver in response to systemic inflammation. It is often elevated in patients with acute ischemia and myocardial infarction (Biasucci et al., 1999). Besides being a predictive factor for myocardial infarction or cerebrovascular accident (Ridker et al., 1997), CRP actively favors atherosclerosis, promoting endothelial activation and macrophage recruitment (Zhong et al., 2006).

The physiological actions of CRP result in a decreased release of nitric oxide and increased expression of IL-6 and IL-8, vascular cellular adhesion of molecules, and ICAM-1 (Zhong et al., 2006). Besides this, the authors also observed that CRP increases the expression of the receptor for AGEs, which is thought to speedup the atherogenesis process.

An evaluation of alcohol consumption over a 12-month period suggested that CRP also shows a U-shaped

association with alcohol consumption (Raum et al., 2006). In this study, the lowest levels of CRP were observed with a consumption of less than 16 g a day. It should be pointed out that the aforementioned study of the effect of alcohol on cardiovascular risk (Maraldi et al., 2006) did not show a mediating effect of CRP on the association between moderate consumption and cardiovascular risk.

Although CRP is influenced by long-term alcohol consumption, drinking in the previous 24 h was not associated with any change in the levels of this marker (Raum et al., 2007).

Hemostatic factors

One of the mechanisms by which moderate alcohol consumption could reduce cardiovascular risk would be by inhibiting platelet reactivity (Belleville, 2002). In this sense, alcohol consumption has been associated with a favorable thrombolytic pattern, protecting against cardiovascular risk (Salem and Laposata, 2005). Moderate alcohol consumption decreases platelet aggregation, increase fibrinolytic activity, and reduce fibrinogen levels (Abou-Agag et al., 2005; Salem and Laposata, 2005). On the other hand, heavy alcohol intake is associated with lower fibrinolytic capacity and a more procoagulant state, with a rise in the plasma levels of factor VII, fibrinogen, and viscosity (Lee and Lip, 2003).

The main determining factors of fibrinolysis are the balance between the tissue plasminogen activating factor and the plasminogen activator inhibitor (PAI), both of them derived from the endothelium. Alcohol consumption appears to elevate these two factors (Puddey et al., 2001). It is possible that the amount of alcohol consumed determines whether these modifications would be predominantly associated with improvement or worsening of endothelial function (Puddey et al., 2001). There is evidence that alcohol acts simultaneously to activate and inhibit platelet function. In this sense, ethanol could be a partial platelet-activating factor (Salem and Laposata, 2005), with partial degranulation allowing the continuous circulation of platelets with an altered function.

The Von Willebrand factor, which is synthetized by the endothelial cells, acts on platelet adhesion and aggregation, and an elevated plasma level suggests endothelial damage (Raitakari and Celermajer, 2000). Alcohol consumption in turn appears to lower the levels of the Von Willebrand factor (Kumari et al., 2000).

A review of the antiatherogenic potential of red wine suggested the existence of an additional benefit (besides alcohol itself) of the phenolic components of red wine over the processes involved in the beginning, progression, and breakdown of atherosclerotic plaques (Szmitko and Verma, 2005). Moderate alcohol consumers could have lower fibrinogen levels, Von Willebrand factor, and factor VII as already mentioned. On the other hand, wine consumers would also have a reduction in the levels of the antigen that inhibits the plasminogen activator (PAI-1-Ag), leading to a reduction in hemostasis (Szmitko and Verma, 2005).

Gender and ethnic differences

Most studies on the effects of ethanol on endothelial function were conducted in men in Western populations. However, alcohol consumption is very common among women, and nearly one-third of the alcohol-dependent individuals in the United States and Australia are women (Teesson et al., 2006). Studies of women with alcohol dependence in treatment suggest that they often experience greater physiological impairment earlier in their drinking careers (Hommer et al., 2001). Among the reasons for such susceptibility is the lower total body water content of women in comparison to men (Mumenthaler et al., 1999) and a diminished activity of alcohol dehydrogenase, the primary enzyme involved in the metabolism of alcohol (Chrostek et al., 2003). Part of these differences in the response to alcohol in women could be related to endothelial function. Rajasingh et al. (2007) reported the occurrence of endothelial cell dysfunction in alcohol-consuming female rodents, suggesting that ethanol blunts the beneficial effects of estrogen on endothelial cells. However, despite these gender differences, a study designed to compare the associations of drinking frequency and quantity with risk of myocardial infarction in men and women showed similar beneficial effects of moderate drinking in both genders (Mukamal et al., 2005).

Considering possible interactions between genetic factors and alcohol consumption on cardiovascular risk (Hines et al., 2001; Younis et al., 2005), ethnicity should also be considered when analyzing the effects of alcohol on cardiovascular health. There are significant genetic differences in ethanol metabolism between Western and Asian populations, where approximately 50% of Orientals lack the activity of the mitochondrial low-Km aldehyde dehydrogenase and present a flushing reaction after drinking that decrease their risk for alcohol dependence (Yin, 1994). There is evidence that blood pressure and HDL cholesterol are more prone to be affected by drinking in flushers than in non-flushers, at least in patients with diabetes (Wakabayashi and Masuda, 2006).

There are also reports on differences between African-derived and European-derived individuals regarding the effects of alcohol on blood pressure. Steffens et al. (2006) verified that non-white individuals who consumed large daily amounts of ethanol (30 g or more for men or 15 g or more for women) were at higher risk of developing hypertension, replicating similar findings from the "Atherosclerosis Risk in Communities Study" cohort for individuals with an African ancestry (Fuchs et al., 2004). The effect would be protective for whites and harmful for blacks. Furthermore, the protective effect appeared to be more intense among whites who rarely consumed alcohol.

These data suggest the possibility that part of the beneficial effects of alcohol could actually be due to a bias related to the life style of the alcoholic beverage users. Among the possible confounding factors are physical activity and the psychosocial profile. A recent review of data on the putative cardioprotective effect of alcohol concluded that a real association between the consumption of alcoholic beverages and the incidence of coronary artery disease has not yet been unveiled (Fuchs and Chambless).

Taken together, it is possible to infer that gender, ethnicity, lifestyle, and genetic polymorphisms might impact the associations between alcohol consumption and cardiovascular risk. These pharmacogenomic and environmental heterogeneity issues clearly deserve further investigation.

Conclusions

Alcohol has dose-dependent and dual effects on several physiological functions, being associated with beneficial and harmful vascular effects. It is difficult to establish the precise sequence of events involved in the action of alcohol on the vascular endothelium at the current stage of research. However, it is possible to infer a pattern in which the first steps following alcohol consumption involve influences in insulin resistance, lipoproteins, oxidative stress, and production of AGEs. The action of these factors in the vascular endothelium may then influence the levels of several markers related to the J-shaped curve.

This huge volume of evidences has generated a broad discussion on the medical attitude toward the consumption of alcoholic beverages. The truth is that data are not yet sufficient to support the prescription of moderate alcohol consumption (or specifically, red wine) to abstemious patients. First of all, so far it has been impossible to totally exclude life style related biases, gender, and ethnic effects and individual genetic variability that might confound the associations observed between alcohol consumption and cardiovascular risk. Furthermore, large long-term, randomized clinical trials would be needed to evaluate the effects of alcohol. However, the ethical aspects of these studies are doubtful, considering the serious negative consequences of alcohol use (Szmitko and Verma, 2005). In view of this limitation, it will still be necessary to rely on the results of prospective controlled observational studies and laboratory studies on mechanisms of action of ethanol to confirm if it is really helpful for cardiovascular health. Considering the addictive potential of alcohol, the identification of substances that mimic only its beneficial effects is warranted.

References

Abe, H., Kawano, Y., Kojima, S., Ashida, T., Kuramochi, M., Matsuoka, H., and Omae, T. (1994). Biphasic effects of repeated alcohol intake on 24-hour blood pressure in hypertensive patients. Circulation *89*, 2626–2633.

Abou-Agag, L. H., Khoo, N. K., Binsack, R., White, C. R., Darley-Usmar, V., Grenett, H. E., Booyse, F. M., Digerness, S. B., Zhou, F., and Parks, D. A. (2005). Evidence of cardiovascular protection by moderate alcohol: role of nitric oxide. Free Radic. Biol. Med. *39*, 540–548.

Agewall, S., Wright, S., Doughty, R. N., Whalley, G. A., Duxbury, M., and Sharpe, N. (2000). Does a glass of red wine improve endothelial function? Eur. Heart J. *21*, 74–78.

Albano, E. (2006). Alcohol, oxidative stress and free radical damage. Proc. Nutr. Soc. *65*, 278–290.

Albert, C. M., Manson, J. E., Cook, N. R., Ajani, U. A., Gaziano, J. M., and Hennekens, C. H. (1999). Moderate alcohol consumption and the risk of sudden cardiac death among US male physicians. Circulation *100*, 944–950.

Artaud-Wild, S. M., Connor, S. L., Sexton, G., and Connor, W. E. (1993). Differences in coronary mortality can be explained by differences in cholesterol and saturated fat intakes in 40 countries but not in France and Finland. A paradox. Circulation *88*, 2771–2779.

Avogaro, A., Watanabe, R. M., Gottardo, L., de Kreutzenberg, S., Tiengo, A., and Pacini, G. (2002). Glucose tolerance during moderate alcohol intake: insights on insulin action from glucose/lactate dynamics. J. Clin. Endocrinol. Metab. *87*, 1233–1238.

Badia, E., Sacanella, E., Fernandez-Sola, J., Nicolas, J. M., Antunez, E., Rotilio, D., de Gaetano, G., Urbano-Marquez, A., and Estruch, R. (2004). Decreased tumor necrosis factor-induced adhesion of human monocytes to endothelial cells after moderate alcohol consumption. Am. J. Clin. Nutr. *80*, 225–230.

Barter, P. J., Nicholls, S., Rye, K. A., Anantharamaiah, G. M., Navab, M., and Fogelman, A. M. (2004). Antiinflammatory properties of HDL. Circ. Res. *95*, 764–772.

Bau, P. F. D., Bau, C. H. D., Naujorks, A. A., and Rosito, G. A. (2005). Early and late effects of alcohol on blood pressure and endothelial function. Alcohol *37*, 53–58.

Baumann, H., and Gauldie, J. (1994). The acute phase response. Immunol. Today *15*, 74–80.

Bell, R. A., Mayer-Davis, E. J., Martin, M. A., D'Agostino, R. B. Jr., and Haffner, S. M. (2000). Associations between alcohol consumption and insulin sensitivity and cardiovascular disease risk factors: the Insulin Resistance and Atherosclerosis Study. Diabetes Care *23*, 1630–1636.

Belleville, J. (2002). The French paradox: possible involvement of ethanol in the protective effect against cardiovascular diseases. Nutrition *18*, 173–177.

Beulens, J. W., Rimm, E. B., Ascherio, A., Spiegelman, D., Hendriks, H. F., and Mukamal, K. J. (2007). Alcohol consumption and risk for coronary heart disease among men with hypertension. Ann. Intern. Med. *146*, 10–19.

Biasucci, L. M., Liuzzo, G., Grillo, R. L., Caligiuri, G., Rebuzzi, A. G., Buffon, A., Summaria, F., Ginnetti, F., Fadda, G., and Maseri, A. (1999). Elevated levels of C-reactive protein at discharge in patients with unstable angina predict recurrent instability. Circulation *99*, 855–860.

Broadley, A. J., Korszun, A., Abdelaal, E., Moskvina, V., Jones, C. J., Nash, G. B., Ray, C., Deanfield, J., and Frenneaux, M. P. (2005). Inhibition of cortisol production with metyrapone prevents mental stress-induced endothelial dysfunction and baroreflex impairment. J. Am. Coll. Cardiol. *46*, 344–350.

Carty, M. F. (1999). Interleukine-6 as a central mediator of cardiovascular risk associated with chronic inflammation, smoking, diabetes, and visceral obesity: down-regulation with essential fatty acids, ethanol and pentoxifylline. Med. Hypotheses *55*, 465–477.

Castell, J. V., Gomez-Lechon, M. J., David, M., Fabra, R., Trullenque, R., and Heinrich, P. C. (1990). Acute-phase response of human hepatocytes: regulation of acute phase protein synthesis by interleukin-6. Hepatology *12*, 1179–1186.

Chicco, A. J., McCarty, H., Reed, A. H., Story, R. R., Westerlind, K. C., Turner, R. T., and Hayward, R. (2006). Resistance exercise training

attenuates alcohol-induced cardiac oxidative stress. Eur. J. Cardiovasc. Prev. Rehabil. *13*, 74–79.

Chrostek, L., Jelski, W., Szmitkowski, M., and Puchalski, Z. (2003). Gender-related differences in hepatic activity of alcohol dehydrogenase isoenzymes and aldehyde dehydrogenase in humans. J. Clin. Lab. Anal. *17*, 93–96.

Clausell, N., Prado, K. F., and Ribeiro, J. P. (1999). Increased plasma levels of soluble vascular cellular adhesion molecule-1 in patients with chest pain and angiographically normal coronary arteries. Int. J. Cardiol. *68*, 275–280.

Corretti, M. C., Anderson, T. J., Benjamin, E. J., Celermajer, D., Charbonneau, F., Creager, M. A., Deanfield, J., Drexler, H., Gerhard-Herman, M., and Herrington, D., et al. (2002). Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. J. Am. Coll. Cardiol. *39*, 257–265.

Dai, X., Thavundayil, J., Santella, S., and Gianoulakis, C. (2007). Response of the HPA-axis to alcohol and stress as a function of alcohol dependence and family history of alcoholism. Psychoneuroendocrinology *32*, 293–305.

Di Castelnuovo, A., Rotondo, S., Iacoviello, L., Donati, M. B., and De Gaetano, G. (2002). Meta-analysis of wine and beer consumption in relation to vascular risk. Circulation *105*, 2836–2844.

Di Gennaro, C., Biggi, A., Barilli, A. L., Fasoli, E., Carra, N., Novarini, A., Delsignore, R., and Montanari, A. (2007). Endothelial dysfunction and cardiovascular risk profile in long-term withdrawing alcoholics. J. Hypertens. *25*, 273–367.

Djousse, L., Ellison, R. C., McLennan, C. E., Cupples, L. A., Lipinska, I., Tofler, G. H., Gokce, N., and Vita, J. A. (1999). Acute effects of a high-fat meal with and without red wine on endothelial function in healthy subjects. Am. J. Cardiol. *84*, 660–664.

Ferrannini, E., Buzzigoli, G., Bonadonna, R., Giorico, M. A., Oleggini, M., Graziadei, L., Pedrinelli, R., Brandi, L., and Bevilacqua, S. (1987). Insulin resistance in essential hypertension. N. Engl. J. Med. *317*, 350–357.

Friedman, L. A., and Kimball, A. W. (1986). Coronary heart disease mortality and alcohol consumption in Framingham. Am. J. Epidemiol. *124*, 481–489.

Fries, J. W., Williams, A. J., Atkins, R. C., Newman, W., Lipscomb, M. F., and Collins, T. (1993). Expression of VCAM-1 and E-selectin in an in vivo model of endothelial activation. Am. J. Pathol. *143*, 725–737.

Fuchs, F. D. (2005). Vascular effects of alcoholic beverages: is it only alcohol that matters? Hypertension *45*, 851–852.

Fuchs, F. D., and Chambless, L. E. (2007). Is the cardioprotective effect of alcohol real? Alcohol *41*, 399–402.

Fuchs, F. D., Chambless, L. E., Folsom, A. R., Eigenbrodt, M. L., Duncan, B. B., Gilbert, A., and Szklo, M. (2004). Association between alcoholic beverage consumption and incidence of coronary heart disease in whites and blacks: the Atherosclerosis Risk in Communities Study. Am. J. Epidemiol. *160*, 466–474.

Furchgott, R. F., and Zawadzki, J. V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature *288*, 373–376.

Furuya, D. T., Binsack, R., and Machado, U. F. (2003). Low ethanol consumption increases insulin sensitivity in Wistar rats. Braz. J. Med. Biol. Res. *36*, 125–130.

Ghiadoni, L., Donald, A. E., Cropley, M., Mullen, M. J., Oakley, G., Taylor, M., O'Connor, G., Betteridge, J., Klein, N., and Steptoe, A., et al. (2000). Mental stress induces transient endothelial dysfunction in humans. Circulation *102*, 2473–2478.

Goldberg, I. J., Mosca, L., Piano, M. R., and Fisher, E. A. (2001). Wine and your heart. Circulation *103*, 472–475.

Gunzerath, L., Faden, V., Zakhari, S., and Warren, K. (2004). National Institute on Alcohol Abuse and Alcoholism report on moderate drinking. Alcohol. Clin. Exp. Res. *28*, 829–847.

Haak, T., Marz, W., Jungmann, E., Hausser, S., Siekmeier, R., Gross, W., and Usadel, K. H. (1994). Elevated endothelin levels in patients with hyperlipoproteinemia. Clin. Investig. *72*, 580–584.

Hashimoto, M., Kim, S., Eto, M., Iijima, K., Ako, J., Yoshizumi, M., Akishita, M., Kondo, K., Itakura, H., and Hosoda, K., et al. (2001). Effect of acute intake of red wine on flow-mediated vasodilatation of the brachial artery. Am. J. Cardiol. *88*, 1457–1460.

Hines, L. M., Stampfer, M. J., Ma, J., Gaziano, J. M., Ridker, P. M., Hankinson, S. E., Sacks, F., Rimm, E. B., and Hunter, D. J. (2001). Genetic variation in alcohol dehydrogenase and the beneficial effect of moderate alcohol consumption on myocardial infarction. N. Engl. J. Med. *344*, 549–555.

Hommer, D., Momenan, R., Kaiser, E., and Rawlings, R. (2001). Evidence for a gender-related effect of alcoholism on brain volumes. Am. J. Psychiatry *158*, 198–204.

Ignarro, L. J., Buga, G. M., Wood, K. S., Byrns, R. E., and Chaudhuri, G. (1987). Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc. Natl. Acad. Sci. USA *84*, 9265–9269.

Kishore, R., Luedemann, C., Bord, E., Goukassian, D., and Losordo, D. W. (2003). Tumor necrosis factor-mediated E2F1 suppression in endothelial cells: differential requirement of c-Jun N-terminal kinase and p38 mitogen-activated protein kinase signal transduction pathways. Circ. Res. *93*, 932–940.

Klatsky, A. L. (2002). Alcohol and cardiovascular diseases: a historical overview. Ann. N.Y. Acad. Sci. *957*, 7–15.

Klatsky, A. L., Armstrong, M. A., and Friedman, G. D. (1992). Alcohol and mortality. Ann. Intern. Med. *117*, 646–654.

Klatsky, A. L., Armstrong, M. A., and Friedman, G. D. (1997). Red wine, white wine, liquor, beer, and risk for coronary artery disease hospitalization. Am. J. Cardiol. *80*, 416–420.

Kumari, M., Marmot, M., and Brunner, E. (2000). Social determinants of von willebrand factor: the Whitehall II study. Arterioscler. Thromb. Vasc. Biol. *20*, 1842–1847.

Kuulasmaa, K., Tunstall-Pedoe, H., Dobson, A., Fortmann, S., Sans, S., Tolonen, H., Evans, A., Ferrario, M., and Tuomilehto, J. (2000). Estimation of contribution of changes in classic risk factors to trends in coronary-event rates across the WHO MONICA Project populations. Lancet *355*, 675–687.

Lazarus, R., Sparrow, D., and Weiss, S. T. (1997). Alcohol intake and insulin levels. The Normative Aging Study. Am. J. Epidemiol. *145*, 909–916.

Lee, D. H., and Jacobs, D. R. Jr. (2005). Association between serum gamma-glutamyltransferase and C-reactive protein. Atherosclerosis *178*, 327–330.

Lee, K. W., and Lip, G. Y. (2003). Effects of lifestyle on hemostasis, fibrinolysis, and platelet reactivity: a systematic review. Arch. Intern. Med. *163*, 2368–2392.

Levin, E. R. (1995). Endothelins. N. Engl. J. Med. *333*, 356–363.

Luedemann, C., Bord, E., Qin, G., Zhu, Y., Goukassian, D., Losordo, D. W., and Kishore, R. (2005). Ethanol modulation of TNF-alpha biosynthesis and signaling in endothelial cells: synergistic augmentation of TNF-alpha mediated endothelial cell dysfunctions by chronic ethanol. Alcohol. Clin. Exp. Res. *29*, 930–938.

McClain, C. J., Barve, S., Deaciuc, I., Kugelmas, M., and Hill, D. (1999). Cytokines in alcoholic liver disease. Semin. Liver Dis. *19*, 205–219.

McKim, S. E., Gabele, E., Isayama, F., Lambert, J. C., Tucker, L. M., Wheeler, M. D., Connor, H. D., Mason, R. P., Doll, M. A., and Hein, D. W., et al. (2003). Inducible nitric oxide synthase is required in alcohol-induced liver injury: studies with knockout mice. Gastroenterology *125*, 1834–1844.

Maiorano, G., Bartolomucci, F., Contursi, V., Minenna, F. S., Di Mise, R., Palasciano, A., Allegrini, B., Amoruso, M., and Kozakova, M. (1999). Noninvasive detection of vascular dysfunction in alcoholic patients. Am. J. Hypertens. *12*, 137–144.

Maraldi, C., Volpato, S., Kritchevsky, S. B., Cesari, M., Andresen, E., Leeuwenburgh, C., Harris, T. B., Newman, A. B., Kanaya, A., and Johnson, K. C., et al. (2006). Impact of inflammation on the relationship among alcohol consumption, mortality, and cardiac events: the

health, aging, and body composition study. Arch. Intern. Med. *166*, 1490–1497.

Marmot, M. G., Rose, G., Shipley, M. J., and Thomas, B. J. (1981). Alcohol and mortality: a U-shaped curve. Lancet *317*, 580–583.

Mineo, C., Deguchi, H., Griffin, J. H., and Shaul, P. W. (2006). Endothelial and antithrombotic actions of HDL. Circ. Res. *98*, 1352–1364.

Mukamal, K. J., Jensen, M. K., Grønbaek, M., Stampfer, M. J., Manson, J. E., Pischon, T., and Rimm, E. B. (2005). Drinking frequency, mediating biomarkers, and risk of myocardial infarction in women and men. Circulation *112*, 1406–1413.

Mumenthaler, M. S., Taylor, J. L., O'Hara, R., and Yesavage, J. A. (1999). Gender differences in moderate drinking effects. Alcohol Res. Health *23*, 55–64.

Pacher, P., Beckman, J. S., and Liaudet, L. (2007). Nitric oxide and peroxynitrite in health and disease. Physiol. Rev. *87*, 315–424.

Pacher, P., Schulz, R., Liaudet, L., and Szabó, C. (2005). Nitrosative stress and pharmacological modulation of heart failure. Trends Pharmacol. Sci. *26*, 302–310.

Pacher, P., and Szabó, C. (2006). Role of peroxynitrite in the pathogenesis of cardiovascular complications of diabetes. Curr. Opin. Pharmacol. *6*, 136–141.

Pai, J. K., Hankinson, S. E., Thadhani, R., Rifai, N., Pischon, T., and Rimm, E. B. (2006). Moderate alcohol consumption and lower levels of inflammatory markers in US men and women. Atherosclerosis *186*, 113–120.

Palmer, R. M., Ferrige, A. G., and Moncada, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature *327*, 524–526.

Puddey, I. B., Zilkens, R. R., Croft, K. D., and Beilin, L. J. (2001). Alcohol and endothelial function: a brief review. Clin. Exp. Pharmacol. Physiol. *28*, 1020–1024.

Raitakari, O. T., and Celermajer, D. S. (2000). Testing for endothelial dysfunction. Ann. Med. *32*, 293–304.

Rajasingh, J., Bord, E., Qin, G., Ii, M., Silver, M., Hamada, H., Ahluwalia, D., Goukassian, D., Zhu, Y., and Losordo, D. W., et al. (2007). Enhanced voluntary alcohol consumption after estrogen supplementation negates estrogen-mediated vascular repair in ovariectomized mice. Endocrinology *148*, 3618–3624.

Raum, E., Gebhardt, K., Buchner, M., Schiltenwolf, M., and Brenner, H. (2007). Long-term and short-term alcohol consumption and levels of C-reactive protein. Int. J. Cardiol *121*, 224–226.

Renaud, S. C., Gueguen, R., Schenker, J., and d'Houtaud, A. (1998). Alcohol and mortality in middle-aged men from eastern France. Epidemiology *9*, 184–188.

Ridker, P. M., Cushman, M., Stampfer, M. J., Tracy, R., and Hennekens, C. (1997). Plasma concentration of C-reactive protein and risks of developing peripheral vascular disease. Circulation *97*, 425–428.

Ridker, P. M., Hennekens, C. H., Roitman-Johnson, B., Stampfer, M. J., and Allen, J. (1998). Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. Lancet *351*, 88–92.

Rimm, E. B., Williams, P., Fosher, K., Criqui, M., and Stampfer, M. J. (1999). Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. BMJ *319*, 1523–1528.

Rosito, G. A., Fuchs, F. D., and Duncan, B. B. (1999). Dose-dependent biphasic effect of ethanol on 24-h blood pressure in normotensive subjects. Am. J. Hypertens. *12*, 236–240.

Sacanella, E., and Estruch, R. (2003). The effect of alcohol consumption on endothelial adhesion molecule expression. Addict. Biol. *8*, 371–378.

Sacanella, E., Estruch, R., Badia, E., Fernandez-Sola, J., Nicolas, J. M., and Urbano-Marquez, A. (1999). Chronic alcohol consumption increases serum levels of circulating endothelial cell/leucocyte adhesion molecules E-selectin and ICAM-1. Alcohol *34*, 678–684.

Salem, R. O., and Laposata, M. (2005). Effects of alcohol on hemostasis. Am. J. Clin. Pathol. *123(Suppl)*, S96–S105.

Schachinger, V., Britten, M. B., and Zeiher, A. M. (2000). Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation *101*, 1899–1906.

Schafer, C., Parlesak, A., Eckoldt, J., Bode, C., Bode, J. C., Marz, W., and Winkler, K. (2007). Beyond HDL-cholesterol elevation: phospholipid enrichment and shift from HDL3 to HDL2 in alcohol consumers. J. Lipid Res. *48*, 1550–1558.

Senault, C., Betoulle, D., Luc, G., Hauw, P., Rigaud, D., and Fumeron, F. (2000). Beneficial effects of a moderate consumption of red wine on cellular cholesterol efflux in young men. Nutr. Metab. Cardiovasc. Dis. *10*, 63–69.

Shai, I., Pischon, T., Hu, F. B., Ascherio, A., Rifai, N., and Rimm, E. B. (2006). Soluble intercellular adhesion molecules, soluble vascular cell adhesion molecules, and risk of coronary heart disease. Obesity *14*, 2099–2106.

Sillaber, I., and Henniger, M. S. (2004). Stress and alcohol drinking. Ann. Med. *36*, 596–605.

Soardo, G., Donnini, D., Varutti, R., Milocco, C., Basan, L., Espósito, W., Casaccio, D., Isola, M., Soldano, F., and Sechi, L. A. (2006). Effects of alcohol withdrawal on blood pressure in hypertensive heavy drinkers. J. Hypertens. *24*, 1493–1498.

Soardo, G., Donnini, D., Varutti, R., Moretti, M., Milocco, C., Basan, L., Espósito, W., Casaccio, D., Stel, G., and Catena, C., et al. (2005). Alcohol-induced endothelial changes are associated with oxidative stress and are rapidly reversed after withdrawal. Alcohol. Clin. Exp. Res. *29*, 1889–1898.

Spieker, L. E., Hurlimann, D., Ruschitzka, F., Corti, R., Enseleit, F., Shaw, S., Hayoz, D., Deanfield, J. E., Luscher, T. F., and Noll, G. (2002). Mental stress induces prolonged endothelial dysfunction via endothelin-A receptors. Circulation *105*, 2817–2820.

Steffens, A. A., Moreira, L. B., Fuchs, S. C., Wiehe, M., Gus, M., and Fuchs, F. D. (2006). Incidence of hypertension by alcohol consumption: is it modified by race? J. Hypertens. *24*, 1489–1492.

Steinberg, D. (1991). Antioxidants and atherosclerosis: a current assessment. Circulation *84*, 1420–1425.

Steptoe, A., Kunz-Ebrecht, S., Owen, N., Feldman, P. J., Willemsen, G., Kirschbaum, C., and Marmot, M. (2003). Socioeconomic status and stress-related biological responses over the working day. Psychosom. Med. *65*, 461–470.

Szmitko, P. E., and Verma, S. (2005). Antiatherogenic potential of red wine: clinician update. Am. J. Physiol. Heart Circ. Physiol. *288*, H2023–H2030.

Teesson, M., Baillie, A., Lynskey, M., Manor, B., and Degenhardt, L. (2006). Substance use, dependence and treatment seeking in the United States and Australia: a cross-national comparison. Drug Alcohol Depend. *81*, 149–155.

Thorand, B., Baumert, J., Doring, A., Schneider, A., Chambless, L., Lowel, H., Kolb, H., and Koenig, W. (2006). Association of cardiovascular risk factors with markers of endothelial dysfunction in middle-aged men and women. Results from the MONICA/KORA Augsburg Study. Thromb. Haemost. *95*, 134–141.

Touyz, R. M., and Schiffrin, E. L. (2003). Role of endothelin in human hypertension. Can. J. Physiol. Pharmacol. *81*, 533–541.

Tunstall-Pedoe, H., Kuulasmaa, K., Mahonen, M., Tolonen, H., Ruokokoski, E., and Amouyel, P. (1999). Contribution of trends in survival and coronary-event rates to changes in coronary heart disease mortality: 10-year results from 37 WHO MONICA project populations. Monitoring trends and determinants in cardiovascular disease. Lancet *353*, 1547–1557.

Tunstall-Pedoe, H., Vanuzzo, D., Hobbs, M., Mahonen, M., Cepaitis, Z., Kuulasmaa, K., and Keil, U. (2000). Estimation of contribution of changes in coronary care to improving survival, event rates, and coronary heart disease mortality across the WHO MONICA Project populations. Lancet *355*, 688–700.

U.S. Department of Health and Human Services and U.S. Department of Agriculture (2005). Dietary guidelines for Americans, 6th ed., Washington, DC: U.S. Government Printing Office.

Vallance, P., and Chan, N. (2001). Endothelial function and nitric oxide: clinical relevance. Heart *85*, 342–350.

Vapaatalo, H., and Mervaala, E. (2001). Clinically important factors influencing endothelial function. Med. Sci. Monit. *7*, 1075–1085.

Vasdev, S., Gill, V., and Singal, P. K. (2006). Beneficial effect of low ethanol intake on the cardiovascular system: possible biochemical mechanisms. Vasc. Health Risk Manag. *2*, 263–276.

Venkov, C. D., Myers, P. R., Tanner, M. A., Su, M., and Vaughan, D. E. (1999). Ethanol increases endothelial nitric oxide production through modulation of nitric oxide synthase expression. Thromb. Haemost. *81*, 638–642.

Wakabayashi, I., and Masuda, H. (2006). Influence of drinking alcohol on atherosclerotic risk in alcohol flushers and non-flushers of Oriental patients with type 2 diabetes mellitus. Alcohol Alcohol. *41*, 672–677.

Wu, D., Zhai, Q., and Shi, X. (2006). Alcohol-induced oxidative stress and cell responses. J. Gastroenterol. Hepatol. *21*, S26–S29.

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K., and Masaki, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature *332*, 411–415.

Yin, S. J. (1994). Alcohol dehydrogenase: enzymology and metabolism. Alcohol Alcohol. Suppl. *2*, 113–119.

Younis, J., Cooper, J. A., Miller, G. J., Humphries, S. E., and Talmud, P. J. (2005). Genetic variation in alcohol dehydrogenase 1C and the beneficial effect of alcohol intake on coronary heart disease risk in the Second Northwick Park Heart Study. Atherosclerosis *180*, 225–232.

Zhong, Y., Li, S. H., Liu, S. M., Szmitko, P. E., He, X. Q., Fedak, P. W., and Verma, S. (2006). C-reactive protein upregulates receptor for advanced glycation end products expression in human endothelial cells. Hypertension *48*, 504–511.

Zilkens, R. R., Burke, V., Hodgson, J. M., Barden, A., Beilin, L. J., and Puddey, I. B. (2005). Red wine and beer elevate blood pressure in normotensive men. Hypertension *45*, 874–879.

Zilkens, R. R., Rich, L., Burke, V., Beilin, L. J., Watts, G. F., and Puddey, I. B. (2003). Effects of alcohol intake on endothelial function in men: a randomized controlled trial. J. Hypertens. *21*, 97–103.

Capítulo III

Justificativa e objetivos

Justificativa:

Esta Tese é parte de uma série de estudos sobre os efeitos do consumo agudo de álcool em parâmetros cardiovasculares. Os primeiros objetivos foram alvo da Dissertação de Mestrado do autor, e consistiram na avaliação da função endotelial e PA em diferentes momentos após o consumo de álcool. O efeito do álcool sobre alguns parâmetros foi muito intenso, especialmente na confirmação do efeito bifásico do mesmo sobre a PA, o que acabou por originar novas hipóteses de pesquisa a fim de preencher algumas lacunas importantes na área.

Em primeiro lugar, entendemos que o estado atual das pesquisas na área demandava um estudo de revisão amplo sobre os mecanismos subjacentes aos efeitos do álcool sobre a saúde cardiovascular. Havia um volume muito grande de publicações sobre o tema nos últimos anos, tornando necessária uma análise crítica sobre as evidências, a qual veio a constituir o Capítulo II desta Tese.

Entre os artigos originais, o primeiro estudou em um grupo de indivíduos jovens e saudáveis a variação ao longo do dia do diâmetro da artéria braquial e função endotelial, parâmetros fundamentais para os estudos de fisiologia cardiovascular. A maior incidência de eventos cardiovasculares no período da manhã poderia talvez ser explicada por um menor diâmetro ou reatividade vascular pela manhã. Os dois principais estudos anteriores a avaliar os dados sobre o diâmetro contavam com um tamanho amostral muito pequeno (Etsuda e cols., 1999) ou com uma amostra heterogênea e de maior faixa etária (Otto e cols., 2004). Este estudo constitui o Capítulo IV da Tese.

No segundo estudo, investigamos se o potencial arritmogênico do uso de álcool poderia ser explicado por uma influência do mesmo sobre a variabilidade da

freqüência cardíaca (Capítulo V). Novamente, havia uma carência de experimentos controlados avaliando esses parâmetros após o consumo de álcool.

Objetivo Geral:

Investigar o efeito agudo decorrente de ingestão de dose isolada de álcool sobre parâmetros cardiovasculares em indivíduos jovens livres de doença cardiovascular.

Objetivos Específicos:

1- Avaliar em indivíduos jovens e saudáveis a variação em parâmetros cardiovasculares (DAB, FMD, NFMD, PA, FC) em diferentes horários do dia;

2- Investigar se a ingestão aguda de álcool tem um efeito em diferentes horários após consumo sobre vários índices da VFC no domínio do tempo.

Capítulo IV

Diurnal variation of vascular diameter and reactivity in healthy young men

Brazilian Journal of Medical and Biological Research (2008) 41: 500-503
ISSN 0100-879X

Diurnal variation of vascular diameter and reactivity in healthy young men

P.F.D. Bau[1,2], C.H.D. Bau[3], A.A. Naujorks[2], G.A. Rosito[4] and F.D. Fuchs[5]

[1]Departamento de Clínica Médica, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Santa Maria, RS, Brasil
[2]Instituto Cardiovascular, ICARDIO, Santa Maria, RS, Brasil
[3]Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil
[4]Departamento de Medicina Preventiva, Fundação Faculdade Federal de Ciências Médicas de Porto Alegre, Porto Alegre, RS, Brasil
[5]Serviço de Cardiologia, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brasil

Correspondence to: P.F.D. Bau, Rua Appel, 655/903, 97015-030 Santa Maria, RS, Brasil
Fax: +55-3025-1212. E-mail: paulobau@gmail.com

The higher incidence of cardiovascular events in the morning is accompanied by an increased vascular tone. However, there are few published studies designed to evaluate the diurnal variation of vascular and endothelial parameters in healthy subjects. In the present investigation, we evaluated the diurnal variation in brachial artery diameter (BAD), flow-mediated dilation (FMD) and endothelium-independent dilation (NFMD) in a homogeneous sample of healthy non-smoker young men. Fifty subjects aged 20.8 ± 0.3 years (range: 18 to 25 years) were investigated by brachial artery ultrasound. Exclusion criteria were female gender and evidence of clinically significant health problems, including obesity. Volunteers were asked to rest and avoid fat meals as well as alcoholic beverages 48 h before and until completion of the evaluations. BAD, FMD and NFMD were measured at 7 am, 5 pm, and 10 pm and tested by repeated measures ANOVA. BAD was smaller at 7 am (mean ± SEM, 3.8 ± 0.1 mm) in comparison with 5 pm (3.9 ± 0.1) and 10 pm (4.0 ± 0.1 mm; $P < 0.001$). FMD values did not change significantly during the day, while NFMD increased more at 7 am (18.5 ± 1.1%), when compared to 15.5 ± 0.9% at 10 pm and 15.5 ± 0.9% at 5 pm ($P = 0.04$). The physiological state of vasoconstriction after awakening, with preserved capability to dilate in the morning, should be considered to be part of the healthy cardiovascular adaptation before considering later life risk factors and endothelial dysfunction.

Key words: Cardiovascular risk factor; Circadian rhythm; Brachial artery; Vasoconstriction; Vascular biology

Received June 15, 2007. Accepted March 4, 2008

Introduction

Cardiovascular events such as sudden death, stroke and acute coronary syndromes have a higher incidence in the period surrounding awakening (1-3). This vulnerable period has been ascribed to cardiocirculatory (3) and hemostatic (4) adaptations typical of this period of day. Changes in these adaptations have been implicated both in the genesis of atherosclerosis (5) and in the higher incidence of cardiovascular events during the morning period (6).

A plethysmographic study of blood flow and vascular resistance in the forearm indicated that the circadian rhythm of vascular tone is related to increased alpha-sympathetic vasoconstrictor activity in the morning (7). This study was followed by others which analyzed the relationships between vascular and endothelial physiology and the increased frequency of cardiovascular events in the morning. Some of these were Doppler ultrasound studies carried out to determine brachial artery diameter (BAD) and its change after a stimulus, allowing inferences about the endothelial function (8). These measures are the endothelium-dependent flow-mediated dilation (FMD) and endothelium-independent nitroglycerin-mediated dilation (NFMD). Part of these studies identified circadian variations and blunted responses during the morning period in

individuals with (9,10) or without (11) a higher risk of developing cardiovascular events. These findings were not reproduced by others (4,12,13). The inconsistency of findings related to the day-night and time of day variation in BAD and FMD could be secondary to the low statistical power of some studies and to the co-existence of different conditions that may influence vascular function, such as risk factors for vascular disease (12,14), use of medications (14), gender (10,14,15), age (14), phase of the menstrual cycle (10,15), nutrition (14), and environmental temperature (10). Therefore, there is a lack of controlled studies specifically designed to evaluate the circadian variation in these parameters by Doppler ultrasound.

In order to determine which morning variations are physiological and present before the appearance of risk factors at an older age, we addressed the influence of time of day on BAD, FMD and NFMD in a homogeneous sample of healthy young men. If these young subjects present either lower FMD or vasoconstriction in the morning, it can be assumed that such characteristics are not the consequence of other risk factors.

Subjects, Material and Methods

The Ethics Committee of our institution approved the study and all participants agreed to participate after reading a detailed informed consent form. The individuals evaluated were 50 male subjects of the control group of a study on the effects of ethanol on cardiovascular parameters (16).

All volunteers were evaluated at the echocardiography laboratory of the Charity Hospital of Santa Maria. They were asked to abstain from alcohol and other psychoactive substances 48 h before the study. Volunteers arrived at the laboratory at 4 pm and were evaluated by clinical history and physical examination, including the measurement of weight, height, and blood pressure. Afterwards, they rested for 10 min before beginning the first evaluation, done at 5 pm.

BAD and flow velocity measures were obtained according to the guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery (8) using an ultrasound instrument with a 10-MHz linear transducer (Aspen, Acuson Computed Sonography, USA). For each volunteer, optimized images of the brachial artery were obtained above the antecubital fossa of the right arm. This position was marked and all subsequent images were obtained at the same site. Vascular measurements were performed in the supine position in a quiet, temperature-controlled room of the echocardiography laboratory, with continuous electrocardiographic monitoring.

Initially, longitudinal two-dimensional images were acquired. Arterial flow velocity measurements were acquired using a pulsed Doppler signal at 60° to the vessel with the range gate (1.5 mm) in the center of the artery. After the baseline recording of BAD, a cuff was inflated in the forearm to 220 mmHg for 5 min, leading to arterial occlusion. During the procedure, the transducer position was carefully maintained. Following the rapid deflation of the cuff, pulsed Doppler signals were recorded for 15 s, and longitudinal images were assessed after 90 s. Thereafter, a 10-min period was allowed for recovery of the vessel. Longitudinal images were then obtained before and 4 min after the administration of 400 µg of sublingual nitroglycerin spray. Measurements were performed in three consecutive cardiac cycles at the peak of the R wave of the electrocardiogram. The measures were later averaged. All images were recorded on VHS for later analysis. Intraobserver variability, i.e., the mean difference between the measurements for each individual, was <3% for all measurements.

The measurements were repeated at 10 pm and 7 am. A standard low-fat meal was served at 8 pm. After the 10-pm evaluation, volunteers received a snack and were allowed to sleep in the laboratory until 6:30 am. Breakfast was served after the 7-am evaluation. Therefore, in the 7-am and 5-pm evaluations, patients had been fasting for more than 4 h, while the 10-pm evaluation was performed 2 h after a light, low-fat meal.

Data are reported as means ± SEM. The time effect on systolic blood pressure, diastolic blood pressure, heart rate, BAD, FMD, and NFMD variation in the three measurements was evaluated by repeated measures ANOVA. Between-time *post hoc* analyses were performed with Bonferroni adjustment for repeated measures using the SPSS software (Version 12.0; SPSS Inc., USA).

Results

Sample characteristics

The sample consisted of 50 healthy, volunteer, nonsmoker men aged 18-25 years (20.8 ± 0.3 years), with no history of cardiovascular disease or use of medication. Their average body mass index was 23.1 ± 0.3 kg/m^2 (range: 18.5 to 28.7). Table 1 shows that both heart rate and blood pressure were higher at 5 pm.

Vascular measurements

BAD did not differ at 5 pm and 10 pm (3.9 ± 0.1 and 4.0 ± 0.1 mm, respectively), but was significantly smaller at 7 am (3.8 ± 0.1 mm, Figure 1).

Flow-mediated dilation was constant through the day

and night, being 5.3 ± 0.5% at 7 am, 5.1 ± 0.6% at 5 pm, and 5.1 ± 0.6% at 10 pm (repeated measures ANOVA).

A significant increase in NFMD was observed at 7 am (18.5 ± 1.1%), compared to 15.5 ± 0.9% at 5 pm and 15.5 ± 0.9% at 10 pm on the previous day (Figure 2).

Discussion

The present study reports the diurnal variation of vascular diameter, evaluated by vascular ultrasound, of healthy young males. The smaller diameter in the morning accompanied by a normal capability to dilate represents an important feature of the physiological adaptation of these parameters after awakening. When individuals get older and have other risk factors related to endothelial dysfunction, even relatively minor degrees of vasoconstriction in the morning may critically reduce blood flow (7).

The cardiovascular adaptation in the morning, characterized by an increase in vascular tone, alpha-sympathetic vasoconstrictor activity, and catecholamine levels, has been reported for other age groups (7,13). As far as we know, our study is the first Doppler ultrasound study to describe smaller BAD in the morning period, contrasting with findings described by others (6,11). This lower BAD is consistent with the increase in peripheral resistance expected at this time of day. The fact that a lower heart rate accompanied the morning vasoconstriction could be explained by a circadian pattern of cardiac output and total peripheral resistance originating from the day-night pattern in physical activity (17). During the nighttime, blood flow to the skeletal muscles is decreased through local autoregulation, which increases total peripheral resistance and decreases cardiac output compared with daytime (17). Therefore, our results are in accordance with the overall cardiovascular adaptation expected after awakening and assuming the upright position. The interpretation that arteries were constricted at that time, maintaining their physiological capability to dilate, was additionally confirmed by the higher NFMD during the morning period. In fact, previous studies have shown that NFMD is inversely correlated with arterial diameter (18).

Although our protocol included FMD measurements, the results should be interpreted with caution since recent studies (19-21) have suggested the need to correct FMD for the stimulus (shear stress). These studies showed that several risk factors for cardiovascular disease actually impact the shear stress itself, instead of endothelial function. A major issue is that FMD does not always reflect nitric oxide activity (21). Unfortunately, the evaluation of shear stress was not thoroughly addressed in the guidelines followed here (8). This scenario suggests that the clinical significance of time of day variation in FMD reported in previous studies (6,9-11) is controversial.

Considering the time of day differences in BAD, the conclusions associated with endothelial function are difficult to interpret. There is a clear need for more studies specifically designed to evaluate the diurnal and circadian variation in these parameters before definitive conclusions about the morning surge in events can be reached. Most

Table 1. Blood pressure and heart rate at the different times of vascular evaluation.

	7 am	5 pm	10 pm	P
HR (bpm)	62.0 ± 1.1	72.2 ± 1.5	68.8 ± 1.4	<0.001
SBP (mmHg)	111.9 ± 1.3	116.3 ± 1.3	113.6 ± 1.3	0.02
DBP (mmHg)	67.5 ± 1.2	70.9 ± 1.3	65.8 ± 1.2	0.008

Data are reported as mean ± SEM. HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure. In the *post hoc* comparisons (with Bonferroni adjustment), HR, SBP, and DBP were higher at 5 pm (repeated measures ANOVA).

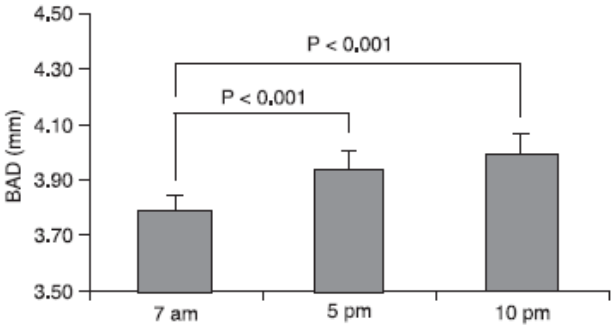


Figure 1. Brachial artery diameter (BAD) at different times of day. Data are reported as means ± SEM. P < 0.001 (repeated measures ANOVA). Significant *post hoc* differences are presented.

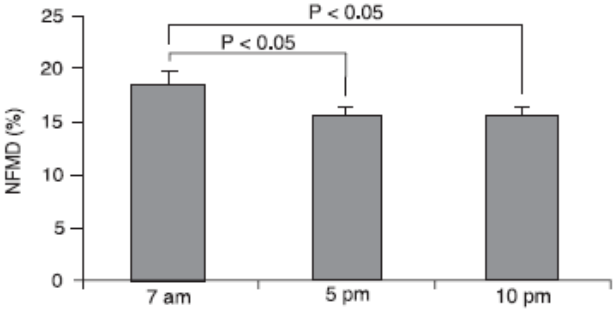


Figure 2. Endothelium-independent dilation (NFMD) measured at different times of day. Data are reported as means ± SEM. P = 0.04 (repeated measures ANOVA). A significant *post hoc* difference is presented.

importantly, the diurnal variation in BAD confounds the results of endothelial function in the early morning. A weakness of the present study was the lack of a more detailed clinical assessment (such as laboratory tests). The young volunteers were considered to be healthy based only on clinical information (anamnesis and physical examination). Another limitation pertains to gender. The fluctuations in estrogen concentrations during the menstrual cycle are reflected in changes in levels of endothelial function markers (15). The present study should be repeated in females in order to determine if there are gender similarities in the diurnal variation in vascular diameter and reactivity.

We demonstrated that, after awakening, young males present a physiological state of vasoconstriction, not accompanied by changes in endothelial function. Changes in this pattern throughout the life span should be taken into account in future studies of the morning peak in cardiovascular events.

References

1. Muller JE, Stone PH, Turi ZG, Rutherford JD, Czeisler CA, Parker C, et al. Circadian variation in the frequency of onset of acute myocardial infarction. *N Engl J Med* 1985; 313: 1315-1322.
2. Cannon CP, McCabe CH, Stone PH, Schactman M, Thompson B, Theroux P, et al. Circadian variation in the onset of unstable angina and non-Q-wave acute myocardial infarction (the TIMI III Registry and TIMI IIIB). *Am J Cardiol* 1997; 79: 253-258.
3. Hu K, Ivanov PC, Hilton MF, Chen Z, Ayers RT, Stanley HE, et al. Endogenous circadian rhythm in an index of cardiac vulnerability independent of changes in behavior. *Proc Natl Acad Sci U S A* 2004; 101: 18223-18227.
4. Shaw JA, Chin-Dusting JP, Kingwell BA, Dart AM. Diurnal variation in endothelium-dependent vasodilatation is not apparent in coronary artery disease. *Circulation* 2001; 103: 806-812.
5. Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* 2000; 101: 1899-1906.
6. Otto ME, Svatikova A, Barretto RB, Santos S, Hoffmann M, Khandheria B, et al. Early morning attenuation of endothelial function in healthy humans. *Circulation* 2004; 109: 2507-2510.
7. Panza JA, Epstein SE, Quyyumi AA. Circadian variation in vascular tone and its relation to alpha-sympathetic vasoconstrictor activity. *N Engl J Med* 1991; 325: 986-990.
8. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002; 39: 257-265.
9. Elherik K, Khan F, McLaren M, Kennedy G, Belch JJ. Circadian variation in vascular tone and endothelial cell function in normal males. *Clin Sci* 2002; 102: 547-552.
10. Gaenzer H, Sturm W, Kirchmair R, Neumayr G, Ritsch A, Patsch J. Circadian variation of endothelium-dependent vasodilatation of the brachial artery as a confounding factor in the evaluation of endothelial function. *Atherosclerosis* 2000; 149: 227-228.
11. Etsuda H, Takase B, Uehata A, Kusano H, Hamabe A, Kuhara R, et al. Morning attenuation of endothelium-dependent, flow-mediated dilation in healthy young men: possible connection to morning peak of cardiac events? *Clin Cardiol* 1999; 22: 417-421.
12. Kawano H, Motoyama T, Yasue H, Hirai N, Waly HM, Kugiyama K, et al. Endothelial function fluctuates with diurnal variation in the frequency of ischemic episodes in patients with variant angina. *J Am Coll Cardiol* 2002; 40: 266-270.
13. Ringqvist A, Caidahl K, Petersson AS, Wennmalm A. Diurnal variation of flow-mediated vasodilation in healthy premenopausal women. *Am J Physiol Heart Circ Physiol* 2000; 279: H2720-H2725.
14. Vapaatalo H, Mervaala E. Clinically important factors influencing endothelial function. *Med Sci Monit* 2001; 7: 1075-1085.
15. Walters J, Skene D, Hampton SM, Ferns GA. Biological rhythms, endothelial health and cardiovascular disease. *Med Sci Monit* 2003; 9: RA1-RA8.
16. Bau PF, Bau CH, Naujorks AA, Rosito GA. Early and late effects of alcohol ingestion on blood pressure and endothelial function. *Alcohol* 2005; 37: 53-58.
17. Veerman DP, Imholz BP, Wieling W, Wesseling KH, van Montfrans GA. Circadian profile of systemic hemodynamics. *Hypertension* 1995; 26: 55-59.
18. Schroeder S, Enderle MD, Baumbach A, Ossen R, Herdeg C, Kuettner A, et al. Influence of vessel size, age and body mass index on the flow-mediated dilatation (FMD%) of the brachial artery. *Int J Cardiol* 2000; 76: 219-225.
19. Mitchell GF, Vita JA, Larson MG, Parise H, Keyes MJ, Warner E, et al. Cross-sectional relations of peripheral microvascular function, cardiovascular disease risk factors, and aortic stiffness: the Framingham Heart Study. *Circulation* 2005; 112: 3722-3728.
20. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. *Circulation* 2007; 115: 1285-1295.
21. Pyke KE, Tschakovsky ME. The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. *J Physiol* 2005; 568: 357-369.

Capítulo V

Acute ingestion of alcohol and cardiac autonomic modulation in healthy volunteers

Acute ingestion of alcohol and cardiac autonomic modulation in healthy volunteers

Paulo FD Bau[1, 2], Ruy S Moraes[3], Claiton HD Bau[4], Elton L Ferlin[3], Guido A Rosito[5], Flávio D Fuchs[3]

[1]Departamento de Clínica Médica, Centro de Ciências da Saúde, Universidade Federal de Santa Maria,
[2]ICARDIO, Santa Maria,
[3]Serviço de Cardiologia, Hospital de Clinicas de Porto Alegre, Porto Alegre, RS, Brazil
[4]Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre,
[5]Departamento de Saúde Coletiva, UFCSPA, Porto Alegre,

Correspondence and reprint requests to:

Paulo F. D. Bau
Departamento de Clínica Médica, UFSM
Rua Appel, 655/903
97015-030 Santa Maria, RS, Brazil
Tel: (55) 3222-0200
Fax: (55) 3223-7300
E-mail: paulobau@gmail.com

Abstract

Cardiac risks of alcohol consumption may be intermediated by its effects over heart rate variability (HRV). Most studies about the effects of alcohol over HRV were observational and did not explore the temporal influence of alcohol ingestion over autonomic modulation. The aim of this study was to verify if an acute ingestion of alcohol has a time-dependent influence over time domain indices of HRV. In a randomized controlled trial, we compared the effect of the ingestion of 60 g of ethanol or placebo over autonomic modulation in healthy men (35 per group), with 18 to 25 years of age, before and during 17 hours after ingestion. Alcohol promoted a fall in standard deviation of all normal R-R intervals (SDNN), root-mean-square of successive differences (RMSSD), and percentage of pairs of adjacent R-R intervals differing by more than 50 ms (PNN50) and in two indices of the three dimensional return map, by a period up to 10 hours after the ingestion of alcohol, accompanied by an increase in heart rate. The indices returned to values similar of the control group 10 hours after ingestion. The effects over HRV indices were attenuated by adjustment for heart rate. The ingestion of alcohol induces a broad cardiovascular adaptation secondary to vagal withdrawal and sympathetic activation that may be responsible for the arrhythmogenic effects of alcohol ingestion.

Key words: cardiovascular risk factor, ethanol, heart rate, arrhythmia.

Introduction

Habitual alcohol consumption may have protective and harmful effects on cardiovascular health. Beneficial effects were described in regard to endothelial function and lipids profile (Vasdev et al., 2006; Bau et al., 2007), but on the other side alcohol consumption is a risk factor for hypertension (Zilkens e cols., 2005). There is also evidence that alcohol may influence autonomic nervous system and heart rhythm regulation. The “holiday heart” syndrome presents as symptomatic atrial fibrillation elicited by acute ingestion of high amounts of alcohol (Ettinger et al., 1978; Nissen and Lemberg, 1984; Rich et al., 1985).

Population-based cohort and cross-sectional studies have evaluated the influence of self-reported alcohol ingestion on autonomic modulation, evaluated by HRV, and its association with cardiovascular outcomes. The findings have not been homogeneous. Janszky et al. (2005) reported an increase in HRV with wine but not with other alcoholic beverages drinking in 102 women with coronary artery disease.. The SAPALDIA study evaluated the effect of alcohol in 1742 randomly selected participants aged $\geq$ 50 years and described a decrease in HRV only with daily moderate alcohol consumption but not with heavier consumption (Felber et al., 2006). In the Whitehall study, based on a sample of 2197 civil servants, the consumption of high amounts of alcohol was associated with higher heart rate and reduced HRV and with the presence of other cardiovascular risk factors (Hemingway et al., 2005). In the CARLA study, which evaluated 1779 elderly individuals with high prevalence of cardiovascular risk factors and cardiovascular disease, alcohol ingestion was not associated to HRV or cardiovascular disease (Greiser et al., 2009). In healthy individuals, it has been demonstrated that alcohol ingestion has acute cardiovascular and autonomic effects that depends on the time elapsed after alcohol

intake. It promotes a biphasic behavior in blood pressure with an initial decrease followed by a latter increase in systolic and diastolic pressures (Abe et al., 1994; Rosito et al., 1999; Bau et al., 2005). After 45 minutes of the ingestion, there is a decrease in blood pressure, lasting up to four hours, accompanied by increases in muscle sympathetic nerve activity and heart rate and a reduction in baro-reflex sensitivity and in HRV (van de Borne et al., 1997). After 12 hours of the ingestion, there is a raise in blood pressure that lasts for about 6 hours (Rosito et al., 1999). The autonomic influence of a single ingestion of alcohol beyond three hours after ingestion has not been properly evaluated in healthy young individuals.

The aim of this controlled randomized trial was to evaluate early and late effects of an acute ingestion of alcohol on HRV in healthy, nonsmoking young men, using time domain and non linear methods.

Methods

2.1. Volunteers

The study group consisted of 70 healthy young men (18-25 years of age, mean 20.7 $\pm$ 2.4 years), with no history of cardiovascular disease or medication use, and non-smokers, recruited from the general population. The ethics committee of our Institution approved the study and all volunteers signed an informed consent to participate.

2.2. Procedures

All volunteers were asked to abstain from alcohol and other psychoactive substances 48 h before the study. The allocation to the alcohol and control groups was performed with a random seed generator. Investigators and volunteers were

blinded to the content of the drink. The beverage contained citric acid (2.6 g), glucose (35.6 g) and, in 50% of the randomized study group, 60 g of ethanol. Distilled water was added to complete 500 ml. The solution was administered between 5:30 PM and 6 PM.

Volunteers were evaluated by clinical history and physical examination (including weight, height and blood pressure) at arrival at the laboratory and afterwards, a Holter recorder was installed. A more detailed characterization of the sample and the description of the experimental procedures were reported before (Bau et al. 2005, 2008).

HRV

The electrocardiographic recording was performed and analyzed with a DMI Burdick / Cardios Holter System V 6.00 B for approximately 19 hours. An investigator blinded in relation to the experimental groups performed the evaluations. The software distinguishes normal beats from ectopy and artifacts, and builds a time series of normal R-R intervals. HRV was analyzed with time domain indices and three-dimensional return map indices. The following time-domain indices were calculated following the recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996): standard deviation of all normal R-R intervals (SDNN); root-mean-square of successive differences (RMSSD); and percentage of pairs of adjacent R-R intervals differing by more than 50 ms (PNN50). The three-dimensional return map, based on non-linear dynamics, is able to reflect 24 hour sympathetic and vagal modulation (Moraes et al., 2000). The map was constructed plotting normal R-R intervals (RR_n) versus difference between adjacent R-R intervals [(RR_{n+1})-(RR_n)] versus counts. In

short, normal R-R intervals were plotted on the X axis against the difference between adjacent R-R intervals on the Y axis. Whenever superimposition of points occurred, the number of superimposed points was expressed on the Z axis, normalized by the maximum density (Figure 1). A set of indices (P_1, P_2, P_3, MN) was calculated to quantify the resulting three-dimensional images. P_1 was calculated as 100 minus the double of the mean slope between 10% and 90% of maximum density, in the plane that intersects the distribution in its maximum concentration of points, perpendicular to RR_n. To calculate P_2 and P_3, three-dimensional images were displayed as 10 equally spaced contour curves: P_2 was calculated as the maximal longitudinal length, and P_3, as the maximal transversal length of the outermost contour curve. The general index MN was calculated as the product of $P_1.P_2.P_3.10^{-3}$ (Moraes et al., 2000).

2.3. Statistical analysis

Data were analyzed using multivariate analysis of variance (MANOVA) for repeated measurements to assess the effects of alcohol ingestion, time after ingestion, and their interaction. Since the effects of alcohol are complex and nonlinear, we decided to compare the effects in somewhat arbitrarily defined periods, based on pharmacokinetics and on the effects of alcohol over blood pressure: pre-ingestion; 1 to 4 hours after ingestion (high plasma levels of alcohol, with vasodilation and lower blood pressure), 4 to 10 hours after ingestion (vanishing of alcohol) and between 10 and 17 hours after ingestion (after elimination, when there is a late increase in blood pressure). These time periods are consistent with the effect of alcohol on blood pressure (Rosito et al., 1999; Bau et al., 2005). Since the evaluation of HRV by the three-dimensional return map is better suited to evaluate autonomic

modulation parameters for longer periods, the post-ingestion period was not divided in this analysis.

The Kolmogorov-Smirnov test was applied to all variables, but none of them departed significantly from normality. All analyses were performed with the SPSS software (Version 12.0; SPSS Inc., USA).

Results

The groups that ingested alcohol or placebo were very similar in regard to several characteristics, as age (20.6 ± 2.4 years versus 20.9 ± 2.4 years, respectively), baseline mean blood pressure (86.6 ± 9.0 mmHg versus 87.2 ± 7.8 mmHg), and BMI (22.1 $\pm$ 2.0 kg/m^2 versus 23.2 $\pm$ 2.2 kg/m^2).

Figure 2 shows the circadian variation of heart rate and the change of heart rate in the alcohol group, by time and experimental groups, showing that besides the circadian variation of heart rate (time effect), there was an interaction between alcohol consumption and time, with an increase of heart rate after ingestion.

Figure 3 shows the variation of variability indices by time and group. SDNN, RMSSD and PNN50 were significantly lower in participants who ingested alcohol. Table 1 presents the comparison of HRV indices in the different periods after ingestion. As can be seen, the differences between the experimental groups were mostly restricted to the periods between 1 and 4 hours and between 4 and 10 hours after consumption, with the exception of SDNN, which was not different between groups between 1 and 4 hours after consumption. In the period between 10 and 17 hours after consumption the two groups did not differ in any HRV index. With the exception of the between-groups differences on PNN50 between 4 and 10 hours after consumption, the remaining indices lost significance after adjustment for heart

rate.

The comparisons of the indices of HRV in the three-dimensional return map are presented in Table 2. P_1 and MN were lower in the participants who did ingest alcohol, but the differences were no longer significant after adjustment for heart rate.

Discussion

The results of this randomized controlled trial showed that in healthy young subjects the ingestion of 60 g of ethanol increases heart rate and reduces HRV evaluated in time domain indices and with the three-dimensional return map, a method based on non linear dynamics. These findings suggest that alcohol acutely promotes vagal withdrawal and increases sympathetic activity that persists for at least 10 hours after ingestion. The autonomic changes observed may play a relevant mediating role in alcohol-related harmful effects on cardiovascular health.

Koskinen et al. (1994) studied the effect of acute alcohol ingestion on HRV in 12 healthy young male adults during 3 hours. HRV was reduced during the whole study after alcohol ingestion, compared to placebo ingestion, when evaluated with a time domain index (RMSSD) and with baro-reflex sensitivity, obtained with simultaneous blood pressure and heart rate spectral analysis. In the same way, in the present study, the reduction in HRV was observed from the first hour after alcohol ingestion for RMSSD and PNN50, and from the fourth hour for SDNN. The reduction in all time domain indices persisted up to 10 hours. This reduction in time domain indices of HRV suggests that there was vagal withdrawal with alcohol ingestion, since these indices mainly represent vagal modulation to the sinus node (Polanczyk et al., 1998). In the study of van de Borne et al. (1997), the effect of alcohol on the autonomic nervous system was evaluated with muscle sympathetic nerve activity,

heart rate and heart rate spectral analysis during 85 minutes after alcohol ingestion. They observed a substantial increase in sympathetic nerve activity, in heart rate and in the low frequency:high frequency ratio during the whole experiment. As heart rate can be used as the net result of autonomic modulation to the sinus node, and when elevated, it reflects sympathetic predominance, in the present study, the increase in heart rate observed with alcohol ingestion points to an early sympathetic over-activity that persisted up to 10 hours after ingestion.

Because of its construction requirements, the three-dimensional return map is more applicable to analyze longer periods of time. In this study, it was used to analyze the whole experiment, starting before alcohol ingestion. The index P_1, calculated at the maximum concentration of points, reflects sympathetic modulation to the sinus node and is increased by sympathetic blockade with propranolol. The indices P_2 and P_3 are the maximum transversal and longitudinal axes of the distribution and represent vagal modulation to the sinus node. Both are reduced by atropine infusion. The global index MN is the product of $P_1.P_2.P_3.10^{-3}$ and reflects vagal and sympathetic modulation, being reduced by the infusion of propranolol and atropine (Moraes et al., 2000). The lower values of P_1, after alcohol ingestion, indicate an increased sympathetic modulation to the sinus node during the whole period analyzed. Considering that P_1 was calculated during a 19 hour period that also included the period before alcohol ingestion, its reduction does not specify when the increase in sympathetic modulation really occurred. On the other hand, it indicates that this increase in sympathetic modulation was intense enough to change HRV behavior during the whole experiment.

Our results are in accordance with the cross-over study of Rossinen et al. (1997) that evaluated the effect of acute alcohol ingestion on 24-hour HRV, in 17

men and 3 women with coronary artery disease and evidence of myocardial ischemia, using cardiovascular medication (beta blockers, calcium antagonists, etc.). The 24-hour time domain indices SDNN and SDANN (standard deviation of the mean RR interval calculated in 5-minute intervals during 24 hours) were reduced with alcohol ingestion compared to placebo, but RMSSD was not affected by alcohol. Heart rate increased 1 hour after alcohol ingestion and remained elevated during 13 hours compared to placebo. The same alcohol induced autonomic changes described by Rossinen et al. (1997) were observed in our healthy subjects suggesting that the effect of alcohol on HRV and heart rate is not substantially influenced by the presence of myocardial ischemia and the use of cardiovascular drugs.

Most population-based cohort and cross-sectional studies that evaluated the influence of alcohol ingestion on autonomic modulation evaluated HRV during short periods of time and did not report the temporal relationship between alcohol intake and autonomic behavior (Janszky et al., 2005; Felber et al., 2006; Hemingway et al., 2005; Greiser et al., 2009). In the study of Ohira et al (2009), a Japanese population-based investigation, the authors evaluated the self-reported habitual alcohol intake in 539 men using 24 hour ambulatory blood pressure, heart rate and power spectral components of HRV. Although this study did not look at the temporal relationship of habitual alcohol intake with blood pressure and autonomic behaviors, 85.3% of moderate drinkers (alcohol intake 23 to 45 g/d) and 97.5% of heavy drinkers (alcohol intake > 46 g/d) reported daily alcohol consumption, enhancing the possibility of the presence of alcohol effect during the 24-hour Holter recording. Compared with nondrinkers, moderate drinkers and heavy drinkers showed higher mean values of systolic and diastolic pressures during the morning and while awake, higher heart

rate while awake and asleep and higher low frequency:high frequency ratio while asleep. These results point to an increased sympathetic modulation associated to alcohol consumption, especially because of the presence of higher heart rates in moderate and heavy drinkers.

The association between elevated heart rate and higher cardiovascular risk is not fully understood but many epidemiological studies have shown that heart rate predicts cardiovascular and non-cardiovascular mortality. In the general population, higher heart rate values at rest are consistently associated with a worse prognosis, even after controlling for other recognized risk factors such as age, gender, blood pressure and serum cholesterol (Morcet et al., 1999; Heidland and Strauer, 2001). Considering that heavy drinkers may have more than only one alcohol ingestion per day, repeated ingestions could maintain an increased sympathetic activity and chronically maintain elevated heart rates contributing to some of the possible harmful effects of alcohol on cardiovascular health**.**

The conflicting results of the impact of alcohol on HRV (Janszky et al., 2005; Felber et al., 2006; Hemingway et al., 2005; Greiser et al., 2009) could be explained by the duration of the autonomic effect after alcohol ingestion demonstrated in our study. After alcohol ingestion, the reduction in HRV was significant but lasted only 10 hours. In studies that used self-reported habitual alcohol ingestion, the time elapsed since last drinking is not informed. It is possible that in many studies subjects were not under the influence of alcohol during HRV analysis.

The Women's Health Study assessed the association of alcohol and atrial fibrillation in 34715 women followed during 12.4 years. The consumption of more than two drinks a day was associated with an increased risk of atrial fibrillation (Conen et al., 2008). Maki et al. (1998) studied the effects of alcohol drinking in

patients with a history of alcohol-associated atrial fibrillation, and detected an increase in lymphocytic beta-adrenoceptor density. Analysis of HRV with spectral analysis revealed an increase in low-frequency/high-frequency ratio suggesting increased sympathetic activity in patients with atrial fibrilation, but not in controls, after ethanol drinking. In addition, Marcus et al. (2008) demonstrated a significant positive association between alcohol use and atrial flutter (but not atrial fibrillation) in younger patients, maybe through an alcohol-induced shortening of right atrial effective refractory periods.

Our study has some limitations that deserve mention. Only male individuals were investigated and therefore the results cannot be extrapolated to women. There is evidence that women respond differently to alcohol ingestion compared to men (Frost and Vestergaard, 2004). The enrollment of healthy and young individuals aimed to have a more homogeneous sample, limiting the potential for confounding.

In conclusion, the ingestion of a single, relatively high dose of alcohol by healthy young males increases heart rate and reduces HRV. These changes are secondary to an increase in sympathetic modulation and a reduction in vagal modulation of the sinus node. The recurrent autonomic effects of alcohol consumption, particularly at higher amounts by individuals with a pattern of abusive consumption, could be related to the deleterious effects of alcohol over the heart.

References

Abe H, Kawano Y, Kojima S, Ashida T, Kuramochi M, Matsuoka H, Omae, T. Biphasic effects of repeated alcohol intake on 24-hour blood pressure in hypertensive patients. Circulation. 1994;89:2626-2633.

Bau PF, Bau CH, Naujorks AA, Rosito GA, Fuchs FD. Diurnal variation of vascular diameter and reactivity in healthy young men. Braz J Med Biol Res. 2008;41:500-3.

Bau PF, Bau CH, Naujorks AA, Rosito GA. Early and late effects of alcohol ingestion on blood pressure and endothelial function. Alcohol. 2005;37:53-8.

Bau PF, Bau CH, Rosito GA, Manfroi WC, Fuchs FD. Alcohol consumption, cardiovascular health, and endothelial function markers. Alcohol. 2007;41:479-88.

Conen D, Tedrow UB, Cook NR, Moorthy MV, Buring JE, Albert CM. Alcohol consumption and risk of incident atrial fibrillation in women. JAMA 2008;300:2489-96.

Ettinger PO, Wu CF, De La Cruz C Jr, Weisse AB, Ahmed SS, Regan TJ. Arrhythmias and the "Holiday Heart": alcohol-associated cardiac rhythm disorders. Am Heart J. 1978;95:555-62.

Felber Dietrich D, Schindler C, Schwartz J, Barthélémy JC, Tschopp JM, Roche F, von Eckardstein A, Brändli O, Leuenberger P, Gold DR, Gaspoz JM, Ackermann-Liebrich U; SAPALDIA Team. Heart rate variability in an ageing population and its association with lifestyle and cardiovascular risk factors: results of the SAPALDIA study. Europace. 2006;8:521-9.

Frost L, Vestergaard P. Alcohol and risk of atrial fibrillation or flutter: a cohort study. Arch Intern Med. 2004;164:1993-8.

Greiser KH, Kluttig A, Schumann B, Swenne CA, Kors JA, Kuss O, Haerting J, Schmidt H, Thiery J, Werdan K. Cardiovascular diseases, risk factors and short-term heart rate variability in an elderly general population: the CARLA study 2002-2006. Eur J Epidemiol. 2009;24:123-42.

Heidland UE, Strauer BE. Left ventricular muscle mass and elevated heart rate are associated with coronary plaque disruption. Circulation. 2001;104:1477-82.

Hemingway H, Shipley M, Brunner E, Britton A, Malik M, Marmot M. Does autonomic function link social position to coronary risk? The Whitehall II study. Circulation. 2005;111:3071-7.

Janszky I, Ericson M, Blom M, Georgiades A, Magnusson JO, Alinagizadeh H, Ahnve S. Wine drinking is associated with increased heart rate variability in women with coronary heart disease. Heart. 2005;91:314-8.

Koskinen P, Virolainen J, Kupari M. Acute alcohol intake decreases short-term heart rate variability in healthy subjects. Clin Sci (Lond). 1994;87:225-30.

Mäki T, Toivonen L, Koskinen P, Näveri H, Härkönen M, Leinonen H. Effect of ethanol drinking, hangover, and exercise on adrenergic activity and heart rate variability in patients with a history of alcohol-induced atrial fibrillation. Am J Cardiol. 1998 Aug 1;82(3):317-22.

Marcus GM, Smith LM, Whiteman D, Tseng ZH, Badhwar N, Lee BK, Lee RJ, Scheinman MM, Olgin JE. Alcohol intake is significantly associated with atrial flutter in patients under 60 years of age and a shorter right atrial effective refractory period. Pacing Clin Electrophysiol. 2008;31:266-72.

Moraes RS, Ferlin EL, Polanczyk CA, Rohde LE, Zaslavski L, Gross JL, Ribeiro JP. Three-dimensional return map: a new tool for quantification of heart rate variability. Auton Neurosci. 2000;83:90-9.

Morcet JF, Safar M, Thomas F, Guize L, Benetos A. Associations between heart rate and other risk factors in a large French population. J Hypertens. 1999;17(12 Pt 1):1671-6.

Nissen MB, Lemberg L. The "holiday heart" syndrome. Heart Lung. 1984;13:89-92.

Ohira T, Tanigawa T, Tabata M, Imano H, Kitamura A, Kiyama M, Sato S, Okamura T, Cui R, Koike KA, Shimamoto T, Iso H. Effects of habitual alcohol intake on ambulatory blood pressure, heart rate, and its variability among Japanese men. Hypertension. 2009;53:13-9.

Polanczyk CA, Rohde LE, Moraes RS, Ferlin EL, Leite C, Ribeiro JP. Sympathetic nervous system representation in time and frequency domain indices of heart rate variability. Eur J Appl Physiol Occup Physiol. 1998;79:69-73.

Rich EC, Siebold C, Campion B. Alcohol-related acute atrial fibrillation. A case-control study and review of 40 patients. Arch Intern Med. 1985;145:830-3.

Rosito GA, Fuchs FD, Duncan BB. Dose-dependent biphasic effect of ethanol on 24-h blood pressure in normotensive subjects. Am J Hypertens. 1999;12:236-240.

Rossinen J, Viitasalo M, Partanen J, Koskinen P, Kupari M, Nieminen MS. Effects of acute alcohol ingestion on heart rate variability in patients with documented coronary artery disease and stable angina pectoris. Am J Cardiol. 1997;79:487-91.

van de Borne P, Mark AL, Montano N, Mion D, Somers VK. Effects of alcohol on sympathetic activity, hemodynamics, and chemoreflex sensitivity. Hypertension.1997;29:1278-83.

Vasdev S, Gill V, Singal PK. Beneficial effect of low ethanol intake on the cardiovascular system: possible biochemical mechanisms. Vasc Health Risk Manag. 2006;2:263-76.

Zilkens RR, Burke V, Hodgson JM, Barden A, Beilin LJ, Puddey IB. Red wine and beer elevate blood pressure in normotensive men. Hypertension 2005;45:874-9.

Figure Legends

Figure 1. Definition of three-dimensional return map-derived indices. Top: 24-h three-dimensional distribution constructed as RR_n versus [(RR_{n+1})-(RR_n)] x counts; the plane depicted intercepts the distribution across its maximum density; counts represent the number of times each event is repeated, generating superimposition of points. Middle: 24-h distribution viewed along the RR_n axis; P_1 was measured at maximum density along the plane described above; P_1=(100-2 x mean slope); mean slope=tangent of the angle Θ. Bottom: contour curves: P_2 is the maximum longitudinal range and P_3 the maximum transversal range. MN is the product P_1. P_2.P_3.10^{-3}. Source: Moraes et al., 2000.

Figure 2. Heart rate in the groups that ingested alcohol (squares) and placebo (triangles). ANOVA: alcohol effect P=0.005; time effect P<0.001; interaction P=0.01.

Figure 3. Heart rate variability in the groups that ingested alcohol (squares) and placebo (triangles). A. SDNN (standard deviation of all normal R-R intervals). ANOVA: alcohol effect P=0.04; time effect P<0.001; interaction P=0.03. B. RMSSD (root-mean-square of successive differences). ANOVA: alcohol effect P=0.06; time effect P<0.001; interaction P=0.01. C. PNN50 (percentage of pairs of adjacent R-R intervals differing by 50 ms). ANOVA: alcohol effect P=0.004; time effect P<0.001; interaction P<0.001;

Table 1. Acute ingestion of alcohol and hourly heart rate and heart rate variability in the different periods after ingestion (mean ± SD)

	Time since intake									
	Before intake	1-4 h	P	P*	4-10 h	P	P*	10-17 h	P	P*
Heart rate										
Alcohol (n=35)	77 ± 11	80 ± 10	0.001	0.001	70 ± 08	0.001	0.001	69 ± 09	0.19	
Placebo (n=35)	75 ± 10	72 ± 09			63 ± 08			66 ± 09		
SDNN										
Alcohol (n=35)	117 ± 38	94 ± 27	0.09	0.79	109 ± 27	0.002	0.31	144 ± 37	0.31	0.83
Placebo (n=35)	119 ± 38	105 ± 25			131 ± 31			153 ± 29		
RMSSD										
Alcohol (n=35)	57 ± 27	41 ± 16	0.006	0.32	57± 25	0.005	0.37	66 ± 29	0.98	0.34
Placebo (n=35)	60 ± 25	54 ± 23			76 ± 28			66 ± 16		
PNN50										
Alcohol (n=35)	28 ± 16	18 ± 11	0.001	0.15	26 ± 14	<0.001	0.03	36 ± 14	0.14	0.66
Placebo (n=35)	28 ± 15	28 ± 14			33 ± 16			41 ± 10		

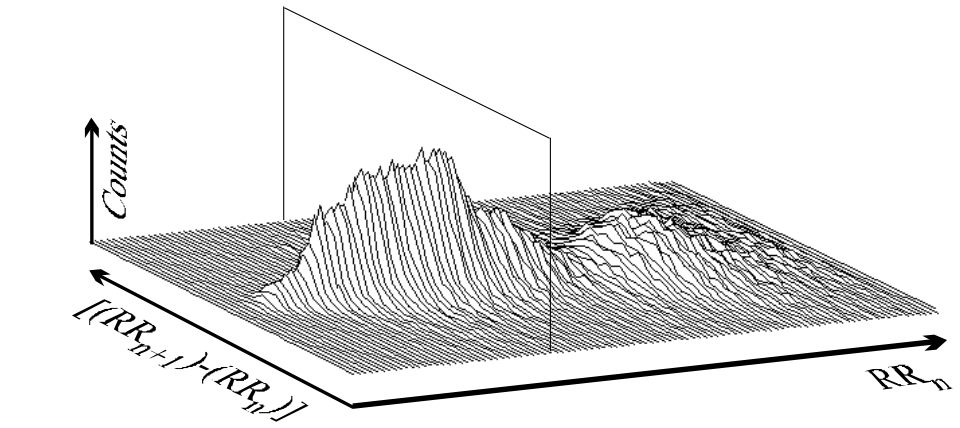
ANOVA results are presented as unadjusted P values or adjusted (P*) for heart rate.
SDNN: standard deviation of all normal R-R intervals; RMSSD: root-mean-square of successive differences; PNN50: percentage of pairs of adjacent R-R intervals differing by 50 ms.

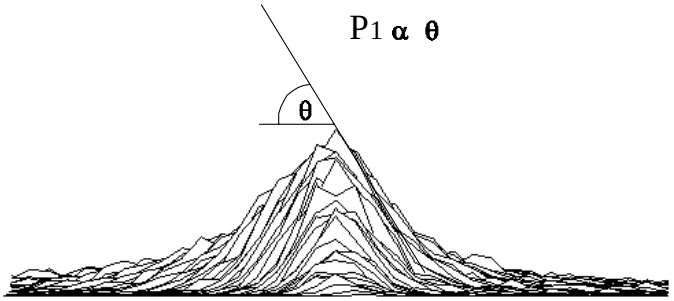
Table 2. The effect of the acute ingestion of alcohol on the return map of heart rate variability (mean ±SD)

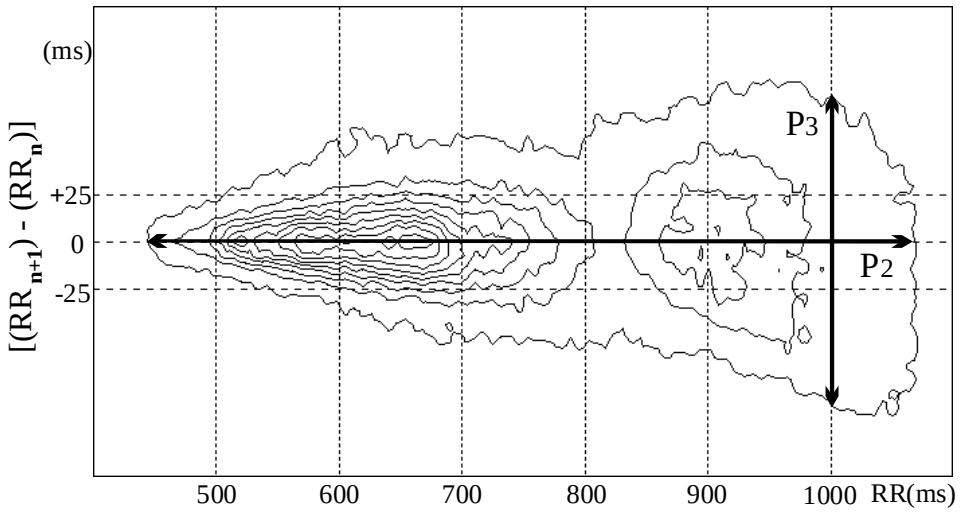
	Experimental groups		P	P*
	Alcohol (n=35)	Placebo (n=35)		
P_1	70 ± 10	76 ± 10	0.02	0.23
P_2	74 ± 11	78 ± 13	0.22	0.97
P_3	125 ± 22	130 ± 27	0.38	0.98
MN	657 ± 216	774 ± 236	0.03	0.58

P for ANOVA unadjusted and adjusted (P*) for heart rate.
Return Map parameters – P_1: 100 minus the double of the mean slope between 10 % and 90 % of maximum density, in the plane that intersects the distribution in its maximum concentration of points, perpendicular to RR_n; P_2: maximal longitudinal length of three-dimensional images; P_3: maximal transversal length of the outermost contour curve; MN: $P_1.P_2.P_3.10^{-3}$

Figura 1







$$MN = P_1 \cdot P_2 \cdot P_3 \cdot 10^{-3}$$

Figura 2

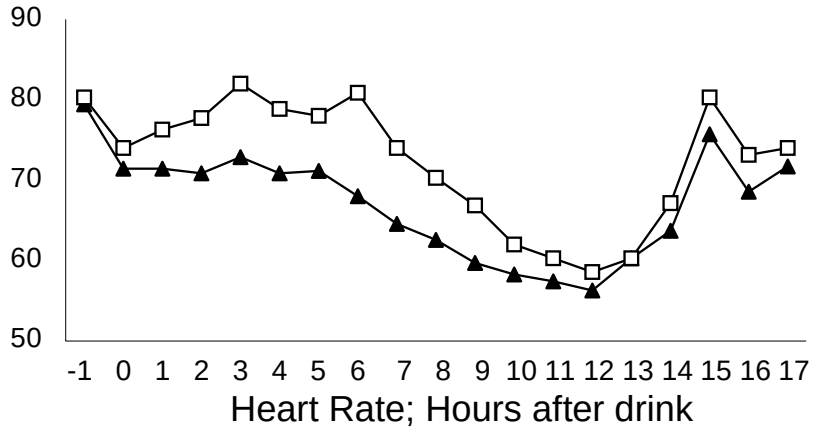
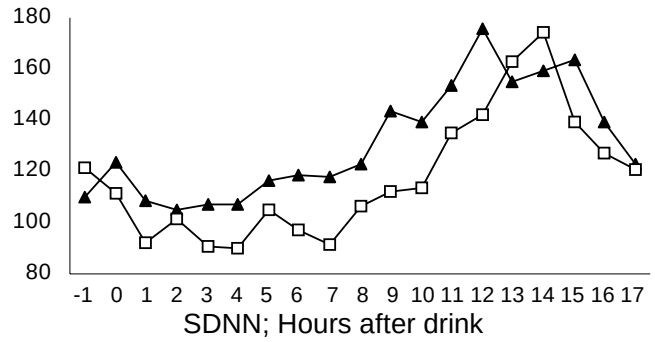
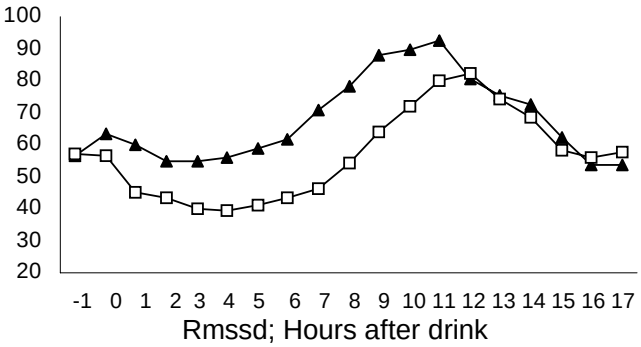

90
80
70
60
50
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Heart Rate; Hours after drink

Figura 3

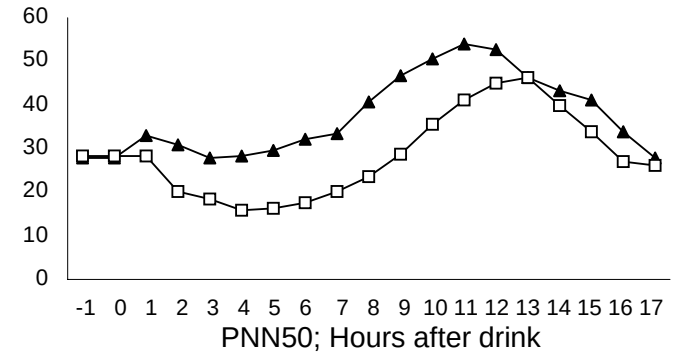
A


180
160
140
120
100
80
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
SDNN; Hours after drink

B


100
90
80
70
60
50
40
30
20
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Rmssd; Hours after drink

C


60
50
40
30
20
10
0
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
PNN50; Hours after drink

Capítulo VI - Discussão

Discussão

Uma discussão mais específica de cada um dos temas desta Tese está contida nos Capítulos II, IV e V. Serão aqui abordados aspectos gerais que não puderam ser considerados nos artigos específicos.

O diâmetro da artéria braquial em diferentes momentos do dia é um parâmetro fisiológico básico para o sistema cardiovascular, e que pode estar sendo negligenciado. Pouquíssimos estudos se voltaram para esse tema. Mais especificamente, conseguimos (Capítulo IV) verificar dados comparáveis em apenas dois estudos anteriores ao nosso. Um deles contava com um tamanho amostral muito pequeno (Etsuda e cols., 1999), e observou uma diferença pequena, não significativa no diâmetro, que poderia ser consistente com os nossos resultados. Já Otto e cols. (2004) estudaram uma amostra heterogênea e de maior faixa etária, não verificando nenhuma diferença entre os horários. Um estudo recente (Kollias e cols., 2008) foi publicado simultaneamente ao nosso, tendo também observado um diâmetro um pouco menor pela manhã, embora a diferença não fosse significativa (P=0.14). No entanto, esse estudo foi realizado em pacientes hipertensos e com sobrepeso.

Se confirmados em estudos com tamanhos amostrais maiores e levando em conta diferentes faixas etárias e presença de fatores de risco, os presentes resultados poderiam ter pelo menos duas conseqüências relevantes. A primeira seria contribuir para o entendimento do maior risco cardiovascular pela manhã, já que esse poderia talvez ser explicado por um menor diâmetro ou reatividade vascular nesse horário. Evidentemente, são necessários mais estudos controlados em uma faixa etária mais avançada, onde existem alterações na função endotelial e outros fatores de risco que poderiam precipitar, pela manhã, um evento coronariano.

Em segundo lugar, seria demonstrada a necessidade de considerar o momento da avaliação na interpretação das medidas de função vascular e endotelial na prática e na pesquisa clínica.

Entre os fatores que podem contribuir na explicação da relação complexa entre o consumo de álcool e o risco cardiovascular encontram-se os seus efeitos sobre a variabilidade da freqüência cardíaca. Demostrou-se que flutuações na freqüência cardíaca relacionadas com o ritmo circadiano endógeno aumentam a vulnerabilidade cardiovascular pela manhã, com um pico em torno das 10 horas (Hu e cols., 2004). Como já foi mencionado, eventos cardiovasculares tais como morte súbita, acidente vascular cerebral e síndromes coronarianas agudas apresentam um pico de incidência pela manhã (Muller e cols., 1985; Cannon e cols., 1997). Assim, alterações da variabilidade da freqüência cardíaca dentro dos limites da normalidade podem ter grande importância sobre o risco cardiovascular, a qual poderia em princípio ser também influenciada pelo uso de álcool.

No artigo que compõe o Capítulo 5 da Tese, avaliamos os efeitos de uma dose aguda de álcool sobre a VFC em um período de até 17h após a ingestão. Os dois únicos experimentos prévios desse tipo (Koskinen e cols., 1994, Vaschillo e cols., 2008) foram restritos a um período de apenas 3 horas, quando apenas uma parte dos efeitos do álcool sobre o sistema cardiovascular poderia ser observada. Ajustando os dados para a FC, Koskinen e cols. (1994) verificaram que o RMSSD mantinha-se ligeiramente diminuído no grupo que consumiu álcool. Já Vaschillo e cols. (2008) não ajustaram os dados para a FC, observando uma redução significativa da VFC que em princípio poderia ser melhor explicada pelo aumento na FC.

Nós verificamos uma redução em vários parâmetros da VFC durante as primeiras 10 horas após o consumo, que consideramos em grande parte atribuível

ao aumento na FC, já que as curvas representativas da VFC e FC eram simetricamente opostas. Dessa maneira, os efeitos do álcool sobre a modulação autonômica parecem resultar de efeitos hemodinâmicos substanciais que levam a uma ativação reflexa do sistema nervoso simpático, com um aumento na freqüência cardíaca. Novas investigações são necessárias com o objetivo de compreender os possíveis mecanismos mediadores da relação entre essa adaptação cardiovascular e os efeitos arritmogênicos e hipertensivos do consumo de álcool.

O consumo intenso de álcool aumenta sem dúvidas a mortalidade por várias causas (Friedman e Kimball, 1986; Marmot e cols., 1981; Renaud e cols., 1998). Portanto, existe uma necessidade de caracterizar detalhadamente esses efeitos, tendo em conta a grande freqüência de consumo na população. Já o consumo moderado de álcool tem efeitos mais complexos, e é alvo de maior discussão. Há evidências sugestivas de que o uso regular, em quantidade pequena a moderada, poderia conferir uma pequena proteção contra eventos arteriais coronarianos e acidentes cerebrais isquêmicos (Gunzerath e cols., 2004). No entanto, mesmo doses baixas poderiam predispor a um aumento na PA em alguns grupos de indivíduos. São necessários mais estudos comparando os efeitos sobre a PA e VFC em normotensos e hipertensos, homens e mulheres, e também levando em conta a faixa etária.

Por outro lado, não é possível afastar-se vieses relacionados com o estilo de vida nos estudos que apontam um efeito benéfico (Fuchs e Chambless, 2007), além de efeitos diferenciais relacionados com o gênero, etnia e variabilidade genética. Os dados obtidos em uma coorte dinamarquesa (Suadicani e cols., 2008) sugeriram que o efeito do consumo de vinho nos riscos de doença cardíaca isquêmica e mortalidade por todas as causas dependeria dos grupos sanguíneos ABO. Os autores pautaram a sua conclusão em nosso artigo de revisão (Bau e cols., 2007),

no sentido de que as inconsistências observadas sobre o efeito do álcool na saúde cardiovascular poderiam ser explicadas por diferenças em frequências gênicas entre populações estudadas. Em princípio, o grupo sanguineo ABO não é um mediador plausível para as relações entre o álcool e a mortalidade. São necessários mais estudos testando interações entre genes com efeito concreto no sistema cardiovascular e o uso de álcool, sobre desfechos relevantes.

O grande volume de evidências sobre o efeito do álcool no sistema cardiovascular tem gerado uma ampla discussão sobre a atitude médica com relação ao consumo de bebidas alcoólicas. É certo que há efeitos deletérios relacionados com o uso do álcool em vários aspectos do sistema cardiovascular, incluindo a PA e um potencial arritmogênico. Além disso, como já foi mencionado o álcool tem conseqüências negativas em muitos outros aspectos, principalmente no âmbito da psiquiatria. Portanto, não é prudente apoiar a prescrição de um consumo moderado de álcool para pacientes abstêmios. Do ponto de vista estritamente técnico, há evidências de que o álcool pode levar a uma redução da mortalidade cardiovascular, mas sob parâmetros muito estreitos e que representam uma fração limitada das situações de consumo. Estes dados podem ter levado muitos médicos e cientistas a um grau de entusiasmo e permissividade com o consumo, especialmente de vinho, que talvez sejam muito negativos para a sociedade como um todo.

Referências bibliográficas da discussão

Bau PF, Bau CH, Rosito GA, Manfroi WC, Fuchs FD. Alcohol consumption, cardiovascular health, and endothelial function markers. Alcohol. 2007;41:479-488.

Cannon CP, McCabe CH, Stone PH, Rogers WJ, Schactman M, Thompson BW, Pearce DJ, Diver DJ, Kells C, Feldman T, Williams M, Gibson RS, Kronenberg MW, Ganz LI, Anderson HV, Braunwald E. The electrocardiogram predicts one-year outcome of patients with unstable angina and non-Q wave myocardial infarction: results of the TIMI III Registry ECG Ancillary Study. Thrombolysis in Myocardial Ischemia. J Am Coll Cardiol. 1997;30:133-140.

Etsuda H, Takase B, Uehata A, Kusano H, Hamabe A, Kuhara R, Akima T, Matsushima Y, Arakawa K, Satomura K, Kurita A, Ohsuzu F. Morning attenuation of endothelium-dependent, flow-mediated dilation in healthy young men: possible connection to morning peak of cardiac events? Clin Cardiol. 1999;22:417-421.

Friedman LA, Kimball AW. Coronary heart disease mortality and alcohol consumption in Framingham. Am J Epidemiol. 1986;124:481-489.

Fuchs FD, Chambless LE. Is the cardioprotective effect of alcohol real? Alcohol. 2007;41:399-402.

Gunzerath, L., Faden, V., Zakhari, S., Warren, K. National Institute on Alcohol Abuse and Alcoholism report on moderate drinking. Alcohol. Clin. Exp. Res. 2004;28:829-847.

Hu K, Ivanov PCh, Hilton MF, Chen Z, Ayers RT, Stanley HE, Shea SA. Endogenous circadian rhythm in an index of cardiac vulnerability independent of changes in behavior. Proc Natl Acad Sci U S A. 2004;101:18223-18227.

Kollias GE, Stamatelopoulos KS, Papaioannou TG, Zakopoulos NA, Alevizaki M,

Alexopoulos GP, Kontoyannis DA, Karga H, Koroboki E, Lekakis JP, Papamichael CM. Diurnal variation of endothelial function and arterial stiffness in hypertension. J Hum Hypertens. 2009 Feb 26. [Epub ahead of print]

Koskinen P, Virolainen J, Kupari M. Acute alcohol intake decreases short-term heart rate variability in healthy subjects. Clin Sci (Lond). 1994;87:225-230.

Marmot MG, Rose G, Shipley MJ, Thomas BJ. Alcohol and mortality: a U-shaped curve. Lancet. 1981;317:580-583.

Muller JE, Stone PH, Turi ZG, Rutherford JD, Czeisler CA, Parker C, Poole WK, Passamani E, Roberts R, Robertson T, et al. Circadian variation in the frequency of onset of acute myocardial infarction. N Engl J Med. 1985;313:1315-1322.

Otto ME, Svatikova A, Barretto RB, Santos S, Hoffmann M, Khandheria B, Somers V. Early morning attenuation of endothelial function in healthy humans. Circulation. 2004;109:2507-2510.

Renaud SC, Gueguen R, Schenker J, d'Houtaud A. Alcohol and mortality in middle-aged men from eastern France. Epidemiology. 1998;9:184-188.

Suadicani P, Hein HO, Gyntelberg F. Wine intake, ABO phenotype, and risk of ischemic heart disease and all-cause mortality: the Copenhagen Male Study-a 16-year follow-up. Alcohol. 2008;42:575-582.

Vaschillo EG, Bates ME, Vaschillo B, Lehrer P, Udo T, Mun EY, Ray S. Heart rate variability response to alcohol, placebo, and emotional picture cue challenges: effects of 0.1-Hz stimulation. Psychophysiology. 2008;45:847-858.

ANEXO 1

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Muitos estudos têm demonstrado que o uso regular de álcool, em quantidade pequena a moderada, parece conferir proteção contra eventos arteriais coronarianos e acidentes cerebrais isquêmicos. Pelo contrário, o consumo pesado de álcool aumenta o risco de tais doenças. Esse aparente efeito protetor do álcool em baixas doses pode se dever às suas ações sobre a função do endotélio (que é o tecido de revestimento dos vasos sangüíneos), o que ainda não foi demonstrado de maneira convincente.

Essa pesquisa tem por objetivo avaliar o efeito do álcool sobre o tecido de revestimento dos vasos sangüíneos.

Poderão fazer parte desse estudo pessoas do sexo masculino, com idade entre 18 e 25 anos, que não apresentam hipertensão arterial sistêmica e que ingerem álcool eventualmente.

Os participantes deverão ficar três dias sem ingerir álcool, devem dormir cedo na noite anterior ao experimento e ingerir dieta balanceada. O estudo será realizado no Hospital de Caridade de Santa Maria. Inicialmente serão instalados um aparelho de Holter, que grava os batimentos cardíacos por 24 horas e a MAPA, que monitora a pressão arterial por 24 horas.

Uma hora antes da ingestão da solução em estudo, todos os casos serão avaliados do ponto de vista clínico (com verificação da PA e FC), será retirado sangue em quatro frascos para posterior análise bioquímica e será submetido a ecografia bidimensional com fluxo a cores da artéria braquial direita. Todos os exames são de caráter não-invasivo, não apresentando riscos. A ecografia vascular poderá causar algum desconforto, já que será provocada uma isquemia temporária do antebraço em estudo, havendo melhora rápida e total ao término do exame.

Após, os participantes serão selecionados ao acaso, em dois grupos: um grupo tomará a solução-padrão que é composta de ácido cítrico (2,6 g), glicose (35,6 g) e água, até completar 500 mililitros. O outro grupo tomará 60 gramas de etanol junto com a solução-padrão. A ingestão será por volta das 18 horas. Uma hora após, todos os participantes farão uma refeição (arroz, feijão, purê de batatas, bife, salada de alface e tomate).

Deverão permanecer no local do estudo, sendo que às 22 horas, aproximadamente 4 horas após a ingestão da solução, serão novamente avaliados clinicamente, retiradas novas amostras de sangue e será feita nova ecografia da artéria braquial direita. Os voluntários passarão a noite no Hospital de Caridade Astrogildo de Azevedo, onde se desenvolverá todo o experimento.

No dia seguinte, às 7 horas (13 horas após a ingestão da solução), serão novamente avaliados clinicamente, serão coletadas novas amostras de sangue e uma última ecografia da artéria braquial direita. Após, os pacientes serão liberados, devendo retornar às 16 horas para a retirada do Holter e da MAPA.

Pelo presente Termo de Consentimento Livre e Esclarecido, declaro que autorizo a minha participação nesse projeto de pesquisa, pois fui informado, de forma clara e detalhada, livre de qualquer forma de constrangimento e coerção, dos objetivos, da justificativa, dos procedimentos a que serei submetido, dos riscos, desconfortos e benefícios, assim como das alternativas às quais poderia ser submetido, todos listados acima. Fui igualmente informado:

-da garantia de receber resposta a qualquer esclarecimento ou dúvida acerca dos procedimentos, riscos, benefícios e outros assuntos relacionados com a pesquisa;
-da liberdade de retirar meu consentimento, a qualquer momento, e deixar de participar do estudo, sem que isso traga prejuízo à continuação do meu cuidado e tratamento;
-da garantia de que não serei identificado, quando da divulgação dos resultados e que as informações obtidas serão utilizadas apenas para fins científicos vinculados ao presente projeto de pesquisa;
-do compromisso de proporcionar informação atualizada durante o estudo, ainda que possa afetar a minha vontade de continuar participando;
-da disponibilidade de tratamento médico e indenização, conforme estabelece a legislação, caso existam danos a minha saúde, diretamente causados por essa pesquisa;
-de que, se existirem gastos adicionais, esses serão absorvidos pelo orçamento da pesquisa.

O pesquisador responsável por esse projeto de pesquisa é Paulo Fernando Dotto Bau, fone 05599614676, tendo como orientador o Prof. Dr. Guido Aranha Rosito. Este documento foi revisado e aprovado pelo Comitê de Ética em Pesquisa da UFRGS.

Data ----/----/----

Nome e assinatura do paciente ou voluntário.

Paulo Fernando Dotto Bau
Nome e assinatura do responsável pela obtenção do presente consentimento.

Observação: O presente documento, baseado no item IV das Diretrizes e Normas Regulamentadoras para a pesquisa em Saúde, do Conselho Nacional de Saúde (resolução 196/96), será assinado em duas vias, de igual teor, ficando uma via em poder do paciente e outra com o pesquisador responsável.

ANEXO 2

UFRGS
UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

PRÓ-REITORIA DE PESQUISA PROPESQ

COMITÊ DE ÉTICA EM PESQUISA

RESOLUÇÃO

O Comitê de Ética em Pesquisa da Universidade Federal do Rio Grande do Sul analisou o projeto:

Número:2004254

Título do projeto: Efeito da ingesta aguda de etanol sobre a função endotelial

Investigador(es) principal(ais): Guido Bernardo Aranha Rosito (Pesq. Resp.) / Paulo Fernando dotto Bau

O mesmo foi aprovado pelo Comitê de Ética em Pesquisa da UFRGS, reunião n.25, ata n. 46, por estar adequado ética e metodologicamente e de acordo com a Resolução 196/96 do Conselho Nacional de Saúde.

Porto Alegre, 08 de abril de 2004.

p/ Prof. José Roberto Goldim
Coordenador CEP/UFRGS

ANEXO 3

ELSEVIER

Alcohol 37 (2005) 53–58

ALCOHOL

Early and late effects of alcohol ingestion on blood pressure and endothelial function

Paulo Fernando Dotto Bau[a,b,*], Claiton H. D. Bau[c], Alexandre A. Naujorks[b], Guido A. Rosito[d,e]

[a]*Department of Clinical Medicine, Health Sciences Centre, Federal University of Santa Maria, Rua Appel, 655/903, 97015-030 Santa Maria, RS, Brazil*
[b]*Cardiovascular Institute (ICARDIO), Santa Maria, RS, Brazil*
[c]*Department of Genetics, Institute of Biosciences, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil*
[d]*Internal Medicine Service, Clinical Hospital of Porto Alegre, Porto Alegre, RS, Brazil*
[e]*Department of Public Health, FFFCMPA, Porto Alegre, RS, Brazil*

Received 30 July 2005; received in revised form 12 October 2005; accepted 25 October 2005

Abstract

Previous investigations have shown a biphasic effect of alcohol on blood pressure (BP). However, there are no studies on possible simultaneous influences in endothelial function. This study aims to evaluate the early and late effects of alcohol ingestion on vascular and endothelial function parameters in healthy young men. The diameter of brachial artery (DBA), endothelium-dependent flow-mediated dilatation, endothelium-independent nitroglycerin-mediated dilatation, systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate were measured 30 min before intake, 4 h after intervention (when there is a reported hypotensive effect of alcohol), and after 13 h (subsequent increase in BP). The study group consisted of 100 males aged 18–25 years who were evaluated by brachial artery ultrasound. Subjects were randomized to drink either an alcoholic (60 g of ethanol) or a similar nonalcoholic beverage. Alcohol induced a biphasic effect on SBP and DBP, with a 4-h decrease followed by an increase after 13 h. After 4 h, the alcohol-drinking group presented a DBA increase that was significant at baseline and after hyperemia but not after nitroglycerin administration. There were no DBA differences between the intervention and control groups 13 h after drinking. This study replicates the initial reports of alcohol-induced biphasic alteration in BP. Our results showed that despite the late increase in BP, there were no accompanying changes in endothelial function.

Keywords: Endothelium; Ethanol; Cardiovascular disease; Prevention; Ultrasound

1. Introduction

There is a strong inverse correlation between moderate alcohol consumption (approximately 30 g/day) and coronary mortality (Albert et al., 1999; Belleville, 2002; Gronbaek, 2002; Rimm et al., 1999). However, heavy drinking increases the mortality rate for several conditions, including cirrhosis and cancer (Friedman & Kimball, 1986; Renaud et al., 1998) resulting in a J curve between mortality and alcohol consumption (Klatsky et al., 1992). The improvement in endothelial function is hypothesized to be one of the protective factors for cardiovascular disease among moderate drinkers (Vlachopoulos et al., 2003).

Regular alcohol consumption is associated with higher blood pressure (BP) (Zilkens et al., 2005). The BP response seems to be proportional to the alcohol dose ingested. The effect of alcohol on 24-h BP reveals a dose-dependent biphasic effect (Abe et al., 1994; Rosito et al., 1999), more pronounced with a higher dose (Rosito et al., 1999). A review of studies on the relationship between alcohol and BP showed that alcohol causes specific early and late effects in BP, with a 4-h nadir followed by peak levels after 10 h (McFadden et al., 2005).

Endothelial dysfunction precedes the formation of the atheromatous plaque and has predictive value for the development of cardiovascular diseases (Schachinger et al., 2000). The evaluation of the endothelium-dependent flow-mediated dilatation (FMD) and endothelium-independent nitroglycerin-mediated dilatation (NFMD) with an ultrasound is a validated noninvasive method to measure endothelial function (Corretti et al., 2002). A blunted endothelial response is associated with various risk factors for

* Corresponding author. Department of Clinical Medicine, Health Sciences Centre, Federal University of Santa Maria, Rua Appel, 655/903, 97015-030 Santa Maria, RS, Brazil. Tel.: +55-55-3222-1929; fax: +55-55-3223-7300.
E-mail address: paulobau@icardio.com.br (P.F.D. Bau).

0741-8329/05/$ – see front matter
doi: 10.1016/j.alcohol.2005.10.034

cardiovascular disease (Gaenzer et al., 2000; Vapaatalo & Mervaala, 2001).

Although alcohol abuse was associated with FMD impairment (Maiorano et al., 1999; Puddey et al., 2001), the same does not seem to happen with moderate drinkers (Zilkens et al., 2003). It is clear that the main vascular consequence of an acute dose of alcohol is vasodilatation (Agewall et al., 2000; Hashimoto et al., 2001; Tawakol et al., 2004; Vlachopoulos et al., 2003); however, there is no consensus on its action on FMD. Although Hashimoto et al. (2001) observed a FMD decrease after the acute alcohol use, others did not observe any change in endothelial function (Agewall et al., 2000; Djousse et al., 1999). Another report suggested that moderate alcohol consumption would improve FMD in people with coronary disease (Teragawa et al., 2002). Unfortunately, most of the studies of alcohol effects on endothelial function could be affected by confounding factors. Arterial diameter and endothelial function measurements are extremely vulnerable to variables such as diseases, medication use, atherothrombotic risk factors, gender, age, menstrual cycle, postprandial period, and temperature (Gaenzer et al., 2000; Vapaatalo & Mervaala, 2001). Other components of alcoholic beverages may also influence vascular parameters. Some studies verified an FMD increase due to nonalcoholic substances in wine (Agewall et al., 2000; Hashimoto et al., 2001) and grape juice (Stein et al., 1999). Moreover, considering the fact that baseline diameter of brachial artery (DBA) is increased after alcohol consumption, it is difficult to compare the percentual changes reported in most studies. It would be more informative to compare absolute changes in brachial diameter in response to reactive hyperemia and nitroglycerin. Finally, none of these endothelial function studies controlled for early and late effects of alcohol on BP.

The aim of this study is to evaluate the acute effect of a relatively high dose of alcohol (60 g) on endothelial function in presumably healthy, nonsmoking young men (18–25 years). In this way, we studied the changes produced when there is a reported hypotensive effect of alcohol (after 4 h) and when there is a subsequent increase in BP (after 13 h).

2. Methods

2.1. Study group

This investigation is part of a series of experiments on the cardiovascular effects of alcohol. The study group consisted of 100 healthy young men (18–25 years, mean 20.74 ± 2.36), with no history of cardiovascular disease or medication use, nonsmokers recruited from the general population. The average body mass index (BMI) was $22.93 \pm 2.40\ kg/m^2$ (range 18.50–29.30). The study was approved by the ethics committee of the Federal University of Rio Grande do Sul. All volunteers signed an informed consent. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

2.2. Procedures

All volunteers were asked to abstain from alcohol and other psychoactive substances 48 h before the study. The evaluations were performed in patients in the supine position in a quiet, temperature-controlled room of the echocardiography laboratory, with electrocardiographic monitoring.

After arrival at the laboratory, volunteers were evaluated by clinical history and physical examination (including weight, height, and BP). Afterward, subjects rested for 10 min before the beginning of the first evaluation (5 p.m., 30 min before the ingestion). The time of the evaluation (from 5 p.m. to 7 a.m. in the next day) was defined based on laboratory availability and volunteers' best convenience.

Supine BP was measured using a mercury sphygmomanometer on the left arm. DBA was obtained according to the guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilatation of the brachial artery (Corretti et al., 2002) with an ultrasound machine with a 10-MHz linear transducer (Aspen-Acuson Computed Sonography). For each volunteer, optimized images of the brachial artery were obtained above the antecubital fossa of the right arm. This position was marked so that all subsequent images could be obtained in the same place. The same sonographer, certified for heart and vascular ultrasound, blind to the control and test groups, evaluated all subjects.

Initially, longitudinal bidimensional images were obtained. After the baseline recording of the diameter, a BP cuff was inflated in the forearm to 250 mmHg for 5 min to produce arterial occlusion. During the procedure, the transducer position was carefully maintained. The cuff was rapidly deflated. Following deflation of the cuff, pulsed Doppler signals were recorded for 15 s, and longitudinal images were assessed after 60–90 s. Thereafter, a 10-min period was allowed for recovery of the vessel. Longitudinal images were then obtained before and 4 min after the administration of 400 μg of sublingual nitroglycerin spray (Corretti et al., 2002). All images were recorded for posterior analysis.

The allocation of groups to either an alcohol containing drink or a similar nonalcoholic beverage was performed with a random seed generator. Each day, two volunteers were evaluated. Investigators and volunteers were blind to the content of the drink, with or without alcohol. The amount of alcohol was the one identified by Rosito et al. (1999) as eliciting a more pronounced effect. The solution contained citric acid (2.6 g), glucose (35.6 g), and, in 50% of the randomized study group, 60 g of ethanol. Distilled water was added to make up to 500 ml. The solution was drunk between 5:30 p.m. and 6 p.m.

The measurements were repeated at 10 p.m. (4 h after intervention) and 7 a.m. (after 13 h). Volunteers were asked to rest between 6 p.m. and 10 p.m. A regular, standardized meal was served at 8 p.m. After the 10 p.m. evaluation,

volunteers received a snack and were allowed to sleep until 6:30 a.m. At 7 a.m., subjects were directed to the echocardiography laboratory in the fasting state for the last evaluation.

Briefly, DBA was measured from the anterior to posterior walls according to the guidelines (Corretti et al., 2002) at 5 p.m., 10 p.m., and 7 a.m. Measurements were performed on three consecutive cardiac cycles in the peak of the R wave of the electrocardiogram. The measures were later averaged. Endothelial function was evaluated through ultrasonographic measures of the brachial artery; FMD was induced by hyperemia and NFMD by nitroglycerin.

2.3. Statistical analysis

All results are presented as mean ± standard error of the mean (S.E.M.). The between-groups comparison in the time periods when the biphasic effect of alcohol is plausible (10 p.m., after 4 h, and 7 a.m., after 13 h) was performed by analysis of variance (ANOVA). Considering the variation in BMI, it was included as a covariate. When BMI did not have a significant effect as a covariate (all variables in Table 1 and Fig. 1) it was excluded from the model and the nonadjusted results were presented. BMI was a significant covariate in the analyses of the absolute values of DBA. In this case, it was kept in the model.

We preferred the between-groups one-way ANOVA at each time period because it would be very difficult, in a repeated measures ANOVA, to distinguish precisely between the time of day and intervention effects at each moment, especially considering that alcohol effects are complex and nonlinear in several different outcomes. Pretreatment values were similar in the intervention and control groups because of the random selection of subjects.

3. Results

3.1. Systolic and diastolic blood pressure

Alcohol had a biphasic effect on BP (Fig. 1). The average values (±S.E.M.) of systolic and diastolic blood pressure (SBP and DBP) 4 h after ingestion were smaller in the intervention group than among controls [SBP: 105.18 ± 1.44 mmHg vs. 113.59 ± 1.28 mmHg ($P < .001$), respectively; DBP: 60.14 ± 1.32 mmHg vs. 65.76 ± 1.21 mmHg ($P < .01$), respectively].

The late effects were in the opposite direction. The alcohol-drinking group presented higher average SBP and DBP 13 h after intervention than controls [SBP: 117.50 ± 1.39 mmHg vs. 111.91 ± 1.29 mmHg ($P < .01$), respectively; DBP: 70.98 ± 1.19 mmHg vs. 67.54 ± 1.25 mmHg ($P < .05$), respectively].

3.2. DBA, FMD, and NFMD

The pretreatment average DBAs in the control and intervention groups did not differ. The average DBA 4 h after intake was increased in the group that was given alcohol (4.41 ± 0.06 mm) when compared with the controls (3.99 ± 0.07 mm; $P < .001$). There was no difference 13 h after intake (Fig. 2).

Percent FMD was lower 4 h after alcohol ingestion (2.43% ± 4.27%) when compared to the control group (5.09% ± 4.53%) ($P = .003$). A corresponding reduction was seen in the percent NFMD (6.30% ± 6.06% and 15.52% ± 6.10%, respectively) ($P < .001$). There were no FMD or NFMD differences 13 h after drinking (Table 1).

Considering that baseline DBA is increased after alcohol consumption, the absolute changes in diameter in response to reactive hyperemia and nitroglycerin are more informative than the percent alterations. After 4 h, the alcohol-drinking group presented a DBA higher than the control group at baseline ($P < .001$) and after hyperemia ($P < .001$) but not after nitroglycerin. There were no DBA differences between the intervention and control groups 13 h after drinking (Fig. 2).

3.3. Heart rate

The average heart rate (HR) 4 h after ingestion was similar in the alcohol-drinking and control groups. There was a significant difference after 13 h, when the group that

Table 1
Early and late effects of alcohol on endothelial function and heart rate (HR)

	Time since intake (h)			P^a	
	Before intake (mean ± S.E.M.)	4 h (mean ± S.E.M.)	13 h (mean ± S.E.M.)	4 h	13 h
FMD (%)					
Alcohol ($n = 50$)	4.22 ± 4.21	2.43 ± 4.27	6.28 ± 5.14	.003	.26
Control ($n = 50$)	5.05 ± 4.18	5.09 ± 4.53	5.27 ± 3.64		
NFMD (%)					
Alcohol ($n = 50$)	13.70 ± 6.10	6.30 ± 6.06	19.00 ± 8.64	<.001	.77
Control ($n = 50$)	15.45 ± 6.54	15.52 ± 6.10	18.51 ± 8.12		
HR (bpm)					
Alcohol ($n = 50$)	72.43 ± 1.59	71.84 ± 1.44	67.08 ± 1.48	.13	.008
Control ($n = 50$)	72.17 ± 1.54	68.76 ± 1.44	61.98 ± 1.15		

Abbreviations: FMD, flow-mediated dilatation; NFMD, nitroglycerin-mediated dilatation; S.E.M., standard error of the mean.
[a]Comparison between the alcohol-drinking and control groups after 4 and 13 h.

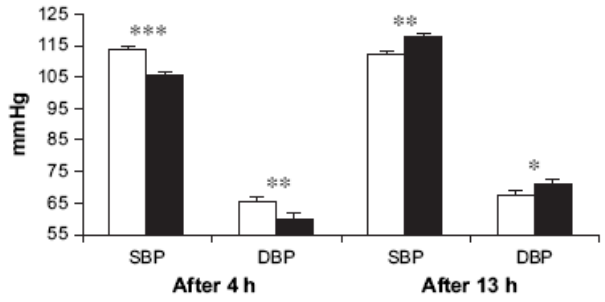


Fig. 1. Systolic (SBP) and diastolic (DBP) blood pressure 4 and 13 h after intervention in the group that received alcohol (black, $n = 50$) and controls (white, $n = 50$). Data are mean ± S.E.M. P values were calculated by analysis of variance. Note the biphasic effect of alcohol. The pretreatment measurements in the intervention and control groups were 114.2 and 115.5 (SBP) and 64.8 and 67.7 (DBP), respectively. $^{***}P < .001$; $^{**}P < .01$; $^{*}P < .05$.

ingested alcohol presented 67.08 ± 1.48 bpm and the control group 61.98 ± 1.15 beats per minute (Table 1).

3.4. Other findings

Eight subjects presented signs of mild intoxication after alcohol consumption, whereas seven presented more important symptoms (dizziness in three; vomit in four).

4. Discussion

This study evaluated the early and late effects of alcohol ingestion on several vascular and endothelial parameters. This investigation replicates the initial reports of alcohol-induced biphasic alteration in BP (Abe et al., 1994; McFadden et al., 2005; Rosito et al., 1999). Most importantly, we evaluated endothelial function parameters 13 h after ingestion, when the early hypotensive and vasodilator effects of alcohol ceased. We did not detect any late effects of ethanol on FMD and NFMD, suggesting that despite the increase in BP, there was no accompanying change in endothelial function.

Alcohol induced a biphasic effect on SBP and DBP, rep
licating previous findings (Abe et al., 1994; McFadde
et al., 2005; Rosito et al., 1999). BP decreased significantl
4 h after intervention and was higher than that in the contro
group after 13 h. The decrease in BP induced by alcohol i
considered to be a response to vasodilatation (Abe et al.
1994), but up to now no known pharmacological mecha
nism convincingly explains the hemodynamic response
to alcohol (Fazio et al., 2004; Vlachopoulos et al., 2003).

Several hypotheses have been raised to explain the lat
increase in BP following alcohol consumption. First, i
might be due to the intrinsic ethanol property of alterin
the actions of cell membrane ion channels (Tawako
et al., 2004). Second, the initial vasodilatation might trigge
a compensatory alcohol-related increase in sympathetic ac
tivity (Randin et al., 1995). Third, the early alcohol-induce
sodium retention might participate in the subsequent BP el
evation (Kawano et al., 2004). There is a clear necessity fo
further research in this field, especially in light of the risk
reduction for cardiovascular events induced by small dose
of alcohol (Fazio et al., 2004).

Parallel to the morning increase in BP, we also observe
a lower morning decrease in HR in the intervention grou
compared to controls. This result is consistent with pre
vious findings of increases in HR following alcohol intak
(Kawano et al., 2004; Rosito et al., 1999; Zilkens et al.
2005). Kawano et al. (2004) suggested that the rise in HR
in the morning following ethanol ingestion the previous
evening might be due to mechanisms related to alcoho
withdrawal, such as activation of the sympathetic nervous
system.

Most investigations on the acute effect of alcohol o
DBA showed a vasodilatation (Agewall et al., 2000
Hashimoto et al., 2001; Tawakol et al., 2004; Vlachopoulos
et al., 2003). Only one study (Djousse et al., 1999) did no
find diameter changes caused by alcohol. A possible ex
planation for the Djousse et al.'s (1999) findings is the fac
that they used a smaller dose (3 ml/kg of wine), nearly 50%
of the one administered in this study, with a high-fat meal

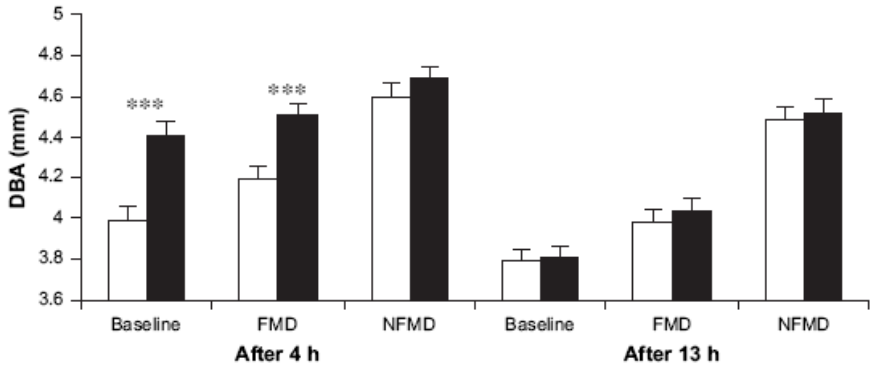


Fig. 2. Diameter of brachial artery (DBA) 4 and 13 h after intervention in the group that received alcohol (black, $n = 50$) and controls (white, $n = 50$). Measurements were done at baseline, after hyperemia (flow-mediated dilatation, FMD) and after nitroglycerin (nitroglycerin-mediated dilatation, NFMD). Data are mean ± S.E.M. P values were calculated by analysis of variance. $^{***}P < .001$.

In this study, the DBA after hyperemia in the group that was given alcohol was higher than in the control group. However, the percent change in diameter was smaller. This result is consistent with the results of Hashimoto et al. (2001), which verified a percent FMD decrease after the acute consumption of Japanese vodka. These results are probably due to the vasodilatation caused by a significant amount of alcohol. Two studies (Agewall et al., 2000; Vlachopoulos et al., 2003) emphasized the possibility that the alcohol-related increase in diameter restricts an additional vasodilatation to be obtained after hyperemia or nitroglycerin. In fact, FMD is inversely correlated with the basal arterial diameter (Hashimoto et al., 2001).

The discrepancies regarding percent changes in FMD a few hours after alcohol ingestion are intriguing. Although we observed a significant decrease, some investigations did not detect any change (Agewall et al., 2000; Djousse et al., 1999; Vlachopoulos et al., 2003). At least part of this difference might be explained by the variation in the type and amounts of alcohol administered. Moreover, most experiments had relatively small study groups, heterogeneous in gender and other variables, with a possible lack of statistical power. Whelan et al. (2004) reported a FMD increase 6 h after wine consumption. This single result might be due to the polyphenols and other substances present in wine, as well as to the fact that the study was performed in coronary disease patients. However, Zilkens et al. (2005) did not detect any long-term effects of red wine or dealcoholized red wine on FMD.

Regarding NFMD, our results agree with the Vlachopoulos et al.'s (2003) findings, showing a significant percent decrease in NFMD a few hours after alcohol consumption (the DBAs in the intervention and control groups were equally enlarged). The alcohol-induced vasodilatation probably prevents further increase in DBA following nitroglycerin (Agewall et al., 2000; Vlachopoulos et al., 2003).

Some limitations of this research should be taken into account. These results are valid only for healthy young men of European descent. Although conduct of the study in a homogeneous sample eliminates confounding, it also limits the relevance of the findings to the onset of acute coronary syndromes, which occur more frequently in older individuals with vascular disease and different arterial reactivity. The results should not be extrapolated to women, elderly, or groups at risk or with cardiovascular disease. Finally, these results are not valid for chronic alcohol abuse, which has already been associated with endothelial dysfunction (Maiorano et al., 1999).

The findings presented here suggest that the consumption of a single, relatively high dose of alcohol does not cause a lasting alteration of the endothelial function in healthy young men. Future studies should verify if frequent alcohol drinking affects endothelial function in the young. Alcohol is consumed by a large fraction of the population in most countries, and moderate consumption has been considered as beneficial. The determination of the type, dosage, and timing of alcohol ingestion associated with endothelial protection or dysfunction would represent an important contribution for the development of public health programs.

Acknowledgments

The Instituto Cardiovascular (ICARDIO) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) funded this research.

References

Abe, H., Kawano, Y., Kojima, S., Ashida, T., Kuramochi, M., Matsuoka, H., & Omae, T. (1994). Biphasic effects of repeated alcohol intake on 24-hour blood pressure in hypertensive patients. *Circulation 89*, 2626–2633.

Agewall, S., Wright, S., Doughty, R. N., Whalley, G. A., Duxbury, M., & Sharpe, N. (2000). Does a glass of red wine improve endothelial function? *Eur Heart J 21*, 74–78.

Albert, C. M., Manson, J. E., Cook, N. R., Ajani, U. A., Gaziano, J. M., & Hennekens, C. H. (1999). Moderate alcohol consumption and the risk of sudden cardiac death among US male physicians. *Circulation 100*, 944–950.

Belleville, J. (2002). The French paradox: possible involvement of ethanol in the protective effect against cardiovascular diseases. *Nutrition 18*, 173–177.

Corretti, M. C., Anderson, T. J., Benjamin, E. J., Celermajer, D., Charbonneau, F., Creager, M. A., Deanfield, J., Drexler, H., Gerhard-Herman, M., Herrington, D., Vallance, P., Vita, J., & Vogel, R. (2002). Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. *J Am Coll Cardiol 39*, 257–265.

Djousse, L., Ellison, R. C., McLennan, C. E., Cupples, L. A., Lipinska, I., Tofler, G. H., Gokce, N., & Vita, J. A. (1999). Acute effects of a high-fat meal with and without red wine on endothelial function in healthy subjects. *Am J Cardiol 84*, 660–664.

Fazio, M., Bardelli, M., Fabris, B., Macaluso, L., Fiammengo, F., Vran, F., Bossi, M., Candido, R., Gerloni, R., & Carretta, R. (2004). Large-artery hemodynamics after acute alcohol administration in young, healthy volunteers. *Angiology 55*, 139–145.

Friedman, L. A., & Kimball, A. W. (1986). Coronary heart disease mortality and alcohol consumption in Framingham. *Am J Epidemiol 124*, 481–489.

Gaenzer, H., Sturm, W., Kirchmair, R., Neumayr, G., Ritsch, A., & Patsch, J. (2000). Circadian variation of endothelium-dependent vasodilatation of the brachial artery as a confounding factor in the evaluation of endothelial function. *Atherosclerosis 149*, 227–228.

Gronbaek, M. (2002). Alcohol, type of alcohol, and all-cause and coronary heart disease mortality. *Ann N Y Acad Sci 957*, 16–20.

Hashimoto, M., Kim, S., Eto, M., Iijima, K., Ako, J., Yoshizumi, M., Akishita, M., Kondo, K., Itakura, H., Hosoda, K., Toba, K., & Ouchi, Y. (2001). Effect of acute intake of red wine on flow-mediated vasodilatation of the brachial artery. *Am J Cardiol 88*, 1457–1460.

Kawano, Y., Abe, H., Kojima, S., Takishita, S., & Matsuoka, H. (2004). Effects of repeated alcohol intake on blood pressure and sodium balance in Japanese males with hypertension. *Hypertens Res 27*, 167–172.

Klatsky, A. L., Armstrong, M. A., & Friedman, G. D. (1992). Alcohol and mortality. *Ann Intern Med 117*, 646–654.

Maiorano, G., Bartolomucci, F., Contursi, V., Minenna, F. S., Di Mise, R., Palasciano, A., Allegrini, B., Amoruso, M., & Kozakova, M. (1999). Noninvasive detection of vascular dysfunction in alcoholic patients. *Am J Hypertens 12*, 137–144.

McFadden, C. B., Brensinger, C. M., Berlin, J. A., & Townsend, R. R. (2005). Systematic review of the effect of daily alcohol intake on blood pressure. *Am J Hypertens 18*, 276–286.

Puddey, I. B., Zilkens, R. R., Croft, K. D., & Beilin, L. J. (2001). Alcohol and endothelial function: a brief review. *Clin Exp Pharmacol Physiol 28*, 1020–1024.

Randin, D., Vollenweider, P., Tappy, L., Jequier, E., Nicod, P., & Scherrer, U. (1995). Suppression of alcohol-induced hypertension by dexamethasone. *N Engl J Med 332*, 1733–1737.

Renaud, S. C., Gueguen, R., Schenker, J., & d'Houtaud, A. (1998). Alcohol and mortality in middle-aged men from eastern France. *Epidemiology 9*, 184–188.

Rimm, E. B., Williams, P., Fosher, K., Criqui, M., & Stampfer, M. J. (1999). Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. *BMJ 319*, 1523–1528.

Rosito, G. A., Fuchs, F. D., & Duncan, B. B. (1999). Dose-dependent biphasic effect of ethanol on 24-h blood pressure in normotensive subjects. *Am J Hypertens 12*, 236–240.

Schachinger, V., Britten, M. B., & Zeiher, A. M. (2000). Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation 101*, 1899–1906.

Stein, J. H., Keevil, J. G., Wiebe, D. A., Aeschlimann, S., & Folts, J. D. (1999). Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation 100*, 1050–1055.

Tawakol, A., Omland, T., & Creager, M. A. (2004). Direct effect of ethanol on human vascular function. *Am J Physiol Heart Circ Physiol 286*, 2468–2473.

Teragawa, H., Fukuda, Y., Matsuda, K., Higashi, Y., Yamagata, T., Matsuura, H., & Chayama, K. (2002). Effect of alcohol consumption on endothelial function in men with coronary artery disease. *Atherosclerosis 165*, 145–152.

Vapaatalo, H., & Mervaala, E. (2001). Clinically important factors influencing endothelial function. *Med Sci Monit 7*, 1075–1085.

Vlachopoulos, C., Tsekoura, D., Tsiamis, E., Panagiotakos, D., & Stefanadis, C. (2003). Effect of alcohol on endothelial function in healthy subjects. *Vasc Med 8*, 263–265.

Whelan, A. P., Sutherland, W. H., McCormick, M. P., Yeoman, D. J., de Jong, S. A., & Williams, M. J. (2004). Effects of white and red wine on endothelial function in subjects with coronary artery disease. *Intern Med J 34*, 224–228.

Zilkens, R. R., Burke, V., Hodgson, J. M., Barden, A., Beilin, L. J., & Puddey, I. B. (2005). Red wine and beer elevate blood pressure in normotensive men. *Hypertension 45*, 874–879.

Zilkens, R. R., Rich, L., Burke, V., Beilin, L. J., Watts, G. F., & Puddey, I. B. (2003). Effects of alcohol intake on endothelial function in men: a randomized controlled trial. *J Hypertens 21*, 97–103.