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IMPACTO DA TERAPIA HORMONAL COM BAIXA DOSE ORAL OU NÃO ORAL SOBRE FATORES DE RISCO CARDIOVASCULAR NA MENOPAUSA

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• Introdução

• Artigo original 1: "Low-dose oral or non-oral hormone therapy: effects on CRP and atrial natriuretic peptide in menopause" (aceito para publicação, *Climateric*, 2014).

• Artigo original 2: "Effects of micronized progesterone added to non-oral estradiol on lipids and cardiovascular risk factors in early menopause: a clinical trial" (publicado em *Lipids in Health and Disease* 2012 11:133).

• Artigo de revisão: "Effects of low-dose postmenopausal hormone therapy on variables related to cardiovascular risk: a systematic review and meta-analyses of randomized clinical trials"

RESUMO

Durante a transição menopausal e a pós-menopausa cerca de 75% das mulheres apresentam sintomas de hipoestrogenismo, tais como fogachos. O emprego de terapia hormonal (TH) para alívio dos sintomas da menopausa está bem estabelecido, mas seus efeitos cardiovasculares (CV) permanecem controversos. Dados de estudos recentes indicam a presença de duas populações distintas quanto aos efeitos CV da TH. Essa diferenciação estaria relacionada principalmente com a idade e o tempo de pós-menopausa. Evidências sugerem também que a presença de fatores de risco cardiovascular antes do início do TH, ou de uma associação de fatores de risco, podem ser determinantes dos efeitos CV do TH. Dose de medicação, via de administração e o tipo de progestogênio utilizado em associação com estrogênio para TH também vem sendo estudados como possíveis fatores relacionados ao impacto CV do TH.

O presente trabalho é composto por: 1) Ensaio clínico randomizado, comparando os efeitos da via oral baixa dose e via não oral sobre variáveis relacionadas com risco CV em uma população de mulheres saudáveis na pós-menopausa recente; 2) Ensaio clínico randomizado, onde foram avaliados os efeitos da adição de progesterona natural micronizada ao estrogênio não oral durante TH em mulheres na pós-menopausa recente; e 3) Revisão sistemática e meta-análise, onde foram sistematicamente buscados todos os artigos com TH baixa dose que avaliassem os efeitos desta terapia sobre variáveis relacionadas com risco cardiovascular: peso, índice de massa corporal, pressão arterial, proteína C reativa e lipídios.

Desenvolvemos ensaio clínico randomizado, cross-over, com objetivo de avaliar os efeitos de dois tipos de tratamento hormonal na menopausa: oral baixa dose, estradiol 1 mg e drospirenona 2 mg diário e não oral, estradiol 17 β gel 1.5 mg (ou nasal 300 mcg) diário e progesterona micronizada vaginal, 200 mg, 14 dias por mês, sobre peptídeo natriurético atrial, variáveis relacionadas com inflamação e função endotelial, perfil antropométrico e metabólico em mulheres na pós-menopausa recente e sem doença clínica evidente. 101 mulheres na pós-menopausa foram alocadas aleatoriamente para iniciar o TH por um dos dois grupos de tratamento: via oral baixa dose (n=50) ou via não oral (n=51). Todas as pacientes utilizaram ambos os

TH de forma seqüencial. Após o primeiro período de 2 a 3 meses de TH a paciente passava para o segundo tratamento, sem período de washout. A avaliação laboratorial foi realizada antes e após cada um dos tratamentos.

A amostra do estudo foi composta por mulheres com média etária de 51 ± 3 anos e tempo de amenorréia de 22 ± 10 meses. Oitenta e seis pacientes concluíram o estudo. Peso e índice de massa corporal não se modificaram, enquanto que a circunferência da cintura reduziu de forma similar em ambos os grupos de tratamento. Colesterol total e LDL-C reduziram após ambos os TH, e triglicerídeos reduziram somente após a TH não oral. Insulina e glicemia de jejum não se modificaram. Não foram observadas modificações nos níveis de fibrinogênio, fator von Willebrand (FvW) e proteína C reativa (PCR) após TH oral. Após TH não oral, observou-se redução significativa de fibrinogênio e FvW. Níveis de PCR não se modificaram. Houve redução do número de pacientes no maior tertil de PCR (alto risco CV) após TH não oral. Essas pacientes passaram a integrar os grupos de risco intermediário e baixo. Níveis de peptídeo natriurético atrial (PNA) mantiveram-se inalterados após os ambos os TH. Não houve modificações significativas na pressão arterial e esta não se correlacionou com valores de PNA. Realizamos análise adicional do TH não oral, quanto às diferenças entre a via nasal e a percutânea e quanto aos efeitos da adição de progesterona natural micronizada ao estrogênio. Não houve diferenças significativas para todas as variáveis estudadas entre a via nasal e a via percutânea. A adição de progesterona natural micronizada não modificou os efeitos metabólicos e CV do estrogênio não oral.

Foi realizada busca sistemática de todos os artigos que incluíssem como TH estrogênio baixa dose e avaliassem os efeitos deste tratamento sobre as variáveis de interesse: peso, índice de massa corporal, pressão arterial, proteína C reativa e lipídeos. Foram consultadas as bases MEDLINE, Cochrane CENTRAL, EMBASE. Foram revisadas todas as referências dos artigos de interesse e revisões e meta-análises no assunto, em busca de artigos relevantes. Após exclusão dos artigos em duplicata, 8610 artigos foram revisados. Destes, 28 artigos foram selecionados para meta-análise. Desta análise foi possível concluir que pacientes em uso de TH baixa dose apresentaram em média menor peso corporal, colesterol total e LDL-C do que não usuárias. A TH baixa dose não apresentou efeitos deletérios sobre demais variáveis estudadas.

Em conclusão, ambos os TH apresentaram efeitos neutros ou benéficos sobre variáveis relacionadas com risco CV em uma população de mulheres na pós-menopausa recente e sem evidência de doença CV. A adição de progesterona natural micronizada não modificou os efeitos do estrogênio não oral. Os resultados da meta-análise sobre TH baixa dose e variáveis relacionadas com risco CV também permitem concluir que a TH baixa dose não exerceu efeitos deletérios sobre lipídeos e pressão arterial, e foi observado um possível efeito benéfico deste tratamento sobre o peso corporal.

Palavras-chave: Menopausa. Tratamento hormonal. Risco cardiovascular. Peptídeo natriurético atrial. Proteína C reativa.

ABSTRACT

During the menopausal transition and postmenopause about 75% of women have symptoms of hypoestrogenism symptoms such as hot flushes. The use of hormone therapy (HT) for relief of menopausal symptoms is well established, but its cardiovascular effects (CV) remain controversial. Data from more recent studies suggest the presence of two distinct populations regarding the cardiovascular effects of HT. This differentiation is related mainly to age and time after menopause. Evidence also suggests that the presence of cardiovascular risk factors before the onset of HT, or a combination of risk factors may be determinants of CV effects of HT. Medication dose, route of administration and type of progestin used in combination with estrogen for HT has also been studied as possible factors related to the CV impact of HT.

This work consists of: 1) Randomized clinical trial, comparing the effects of low dose oral and non-oral route of variables related to CV risk in a population of healthy women in early postmenopausal; 2) A randomized clinical trial, which we assessed the effects of the addition of natural micronized progesterone to non-oral estrogen for HT in women in early postmenopausal; and 3) systematic review and meta-analysis, which were systematically searched all items with low-dose HT to assess the effects of this therapy on variables related to cardiovascular risk: weight, body mass index, blood pressure, C-reactive protein and lipids.

A cross-over, randomized clinical trial was designed in order to evaluate the effects of two types of HT: low dose oral treatment, estradiol oral 1 mg and drospirenone 2 mg, by day and non-oral treatment, estradiol 1.5 mg 17 β gel by percutaneous route (or nasal route 300 mcg) by day and vaginal micronized progesterone, 200 mg/d, 14 days by month on atrial natriuretic peptide, variables associated with inflammation and endothelial function, anthropometric and metabolic variables on early and healthy postmenopausal women. One hundred one women

were randomly allocated to start with one of the treatments: low dose oral treatment (n=50) or non-oral treatment (n=51). At the end the first three months period, the patients were crossed over without washout for an additional three months. Laboratory evaluated were carried before and after oral and non-oral HT.

The sample of the study included postmenopausal women with a mean age of 51 years and duration of amenorrhea of 22±10 months. Eighty-six patients completed the study. Weight and body mass index remained unchanged, while the waist circumference decreased similarly in both treatment groups. Total cholesterol and LDL-cholesterol reduced after both the HT and triglycerides reduced only after non-oral HT. Insulin and fasting glucose did not change. No changes were observed in the levels of fibrinogen, von Willebrand factor (vWF) and C-reactive protein (CRP) after oral HT. After non-oral HT, there was a significant reduction of fibrinogen and vWF. CRP levels did not change. There was a reduction in the number of patients in the highest tertile of CRP (high CV risk) after non-oral HT. These patients have joined the groups of intermediate and low risk. Levels of atrial natriuretic peptide, ANP, were unchanged after both HT. There were no significant changes on blood pressure and did not correlate with values of ANP. We performed additional analysis of non-oral HT, for the differences between nasal and percutaneous and about the effects of addition of natural micronized progesterone to estrogen. There were no significant differences for all the variables studied between the nasal and percutaneously. The addition of micronized natural progesterone did not modify the metabolic and CV effects of non-oral estrogen.

Systematic search of all articles that include as TH low dose estrogen and evaluate the effects of this treatment on the variables of interest was taken: weight, body mass index, blood pressure, C-reactive protein and lipids. The MEDLINE, Cochrane CENTRAL, EMBASE databases were consulted. All references of interest and reviews and meta-analyzes on the subject, in search of relevant articles were reviewed. After removing duplicate articles, 8610 articles were reviewed. Of these, 28 articles were selected for meta-analysis. From this analysis it was concluded that patients using low-dose TH had on average lower body weight, total cholesterol and LDL-C than non-users. The TH low dose showed no deleterious effects on other variables.

In conclusion, low-dose oral and non-oral treatments had neutral or beneficial effects on variables related to CV risk in a population of women in early post-

menopausal and without evidence of CV disease. The addition of micronized natural progesterone did not modify the effects of non-oral estrogen. The results of the meta-analysis of low dose and TH variables related CV risk also showed that the TH low dose did not exert deleterious effects on lipids and blood pressure, and a possible beneficial effect of this treatment on body weight was observed.

Keywords: Menopause. Hormone treatment. Cardiovascular risk. Atrial natriuretic peptide. C-reactive protein.

SUMÁRIO

INTRODUÇÃO

A pós-menopausa é definida como a ausência permanente das menstruações, em decorrência da perda da função folicular ovariana ou remoção cirúrgica dos ovários. A pós-menopausa abrange os estágios precoce e tardio. O estágio precoce define-se como os cinco primeiros anos depois do último sangramento menstrual, e a menopausa tardia como os anos posteriores (Harlow, Gass et al. 2012). A presença de sintomas vasomotores é mais comum na perimenopausa e dentro dos primeiros anos de pós-menopausa, e cerca de 75% das mulheres com mais de 50 anos de idade apresentam, em algum momento, sintomas vasomotores tais como fogachos (Utian 2005). Um estudo na população brasileira, com mulheres em sua maioria caucasianas, classes B e C, da região de São Paulo, estimou em 70% a prevalência de fogachos nesta população. Entre estas mulheres que apresentavam sintomas, 53% classificaram estes sintomas como intensos. Fogachos, insônia e "suores" foram associados fortemente com o climatério (Silva Filho, Baracat et al. 2005).

A terapia hormonal (TH) com estrogênios permanece como o tratamento mais eficaz para os sintomas do hipoestrogenismo (NAMS, 2012) . O tratamento hormonal sistêmico inclui dois componentes principais, o estrogênio e o progestogênio. Como estrogênios, o valerato de estradiol natural ou 17b-estradiol micronizado são frequentemente utilizados na Europa, enquanto os estrogênios conjugados eqüinos (EEC) derivados da urina de éguas prenhes é o produto preferido nos Estados Unidos. Após ingestão oral, a dose equipotente de 2 mg de valerato de estradiol corresponde a aproximadamente 1,5 mg de 17b-estradiol micronizado, 0,625 mg de estrogênios eqüinos conjugados, 50 mg liberados por adesivo, 1,5 mg aplicados como gel percutâneo ou 3,0 mg através de administração nasal (Kiran, Kiran et al. 2004).

Muita importância tem sido atribuída, também, ao tipo e a dose de progestogênio utilizados em combinação com o estrogênio para TH (Canonico, Plu-Bureau et al.2011 ; Sare, Gray et al. 2008). Basicamente, todos os progestogênios têm apenas um efeito em comum, o efeito progestogênico sobre o endométrio, resultando na transformação do endométrio para a fase secretora e na diminuição da espessura endometrial. Os produtos mais comuns são aqueles baseados no esteróide C19

testosterona, os derivados da 17-hidroxiprogesterona, os derivados da 19-norprogesterona, a retroprogesterona, diidrogesterona e a progesterona micronizada. A proteção endometrial é atualmente a única indicação relacionada à menopausa para o uso de progestogênios e pode ser obtida com todos os progestogênios ou com a progesterona natural quando dados seqüencialmente ou continuamente no tratamento hormonal sistêmico. Observa-se ampla diferença entre os progestogênios e seus múltiplos efeitos biológicos, resultantes da habilidade de cada progestogênio ou do seu metabólito em ligar-se a receptores esteróides específicos (Archer 2005).

Os efeitos cardiovasculares (CV) do TH permanecem sendo estudados e são controversos (Khalil 2013). A doença cardiovascular é a principal causa de morte entre as mulheres na pós-menopausa (Grodstein, Manson et al. 2000). Durante a transição menopausal e a pós-menopausa muitas mulheres apresentam ganho de peso e modificações da composição corporal, como aumento de deposição de gordura central (Sternfeld, Wang et al. 2004), modificações em direção a um perfil mais aterogênico, tais como aumento dos triglicerídeos, LDL-C e redução dos níveis de HDL-C (Jenner, Ordovas et al. 1993; de Aloysio, Gambacciani et al. 1999), aumento da pressão arterial (Muchanga, Lepira et al. 2013), e incremento de marcadores relacionados à inflamação crônica (Cioffi, Esposito et al. 2002). Essas modificações são associadas com envelhecimento (El Khoudary, Wildman et al. 2012), mas também com o hipoestrogenismo característico da pós-menopausa (El Khoudary, Wildman et al. 2012 ; Derby, Crawford et al. 2009) e provavelmente estão relacionadas com o aumento de risco cardiovascular que ocorre neste período (Mikkola and Clarkson 2002).

Estudos observacionais associaram à TH com uma redução de 30 a 50% no risco de eventos coronarianos (Grodstein, Manson et al. 2000). No entanto, dados derivados de grandes ensaios clínicos falharam em demonstrar benefício cardiovascular da TH, e demonstraram que a TH pode estar associada com significativo aumento de acidente vascular cerebral isquêmico e outros eventos tromboembólicos (Grady, Wenger et al. 2000; Grady, Herrington et al. 2002; Hendrix, Wassertheil-Smoller et al. 2006).

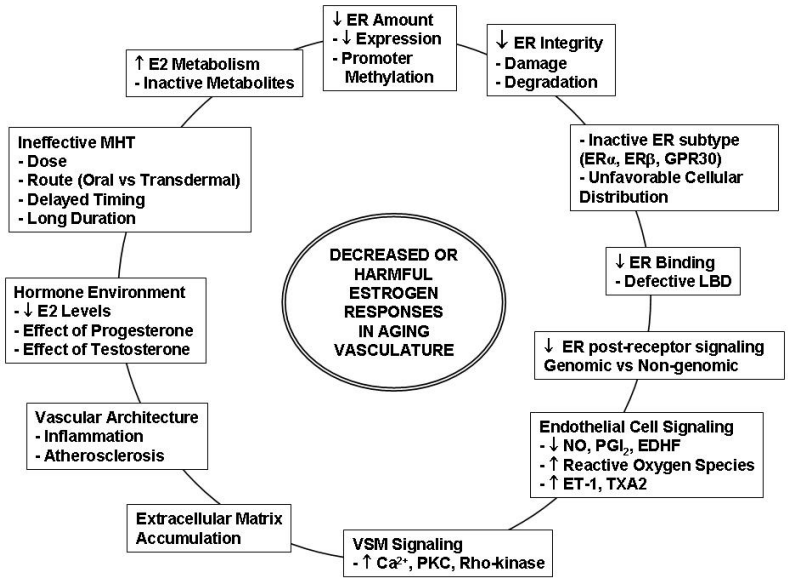
Dados derivados de diversos estudos apontam para a presença de duas populações distintas de mulheres quanto aos efeitos cardiovasculares da TH. Essa diferenciação estaria relacionada principalmente com a idade e o tempo de pós-menopausa (Hodis and Mack 2013), (Manson, Bassuk et al. 2006) (Harman, Naftolin

et al. 2005; Rossouw, Prentice et al. 2007). Duas meta-análises avaliam o impacto CV da TH em mulheres mais jovens e com menos de 10 anos de pós-menopausa (Salpeter, Walsh et al. 2006; Salpeter, Walsh et al. 2006). Nessa população, a TH foi associada com menor risco de doença coronariana (Salpeter, Walsh et al. 2006) e redução da mortalidade total (Salpeter, Walsh et al. 2004). Recente ensaio clínico publicado com 1006 mulheres saudáveis na pós-menopausa precoce, com 42 a 58 anos de idade (Schierbeck, Rejnmark et al. 2012), demonstrou que após 10 anos de randomização para TH, aquelas que receberam TH no período da pós-menopausa recente apresentaram menor risco de morte por todas as causas, infarto agudo do miocárdio e falência cardíaca.

Mecanismos moleculares e celulares suportam a "hipótese do tempo" (tempo de pós-menopausa relacionado com efeitos CV do TH) (Clarkson, Melendez et al.2013), e estariam relacionados com modificações pró-aterogênicas e inflamatórias no sistema cardiovascular, intensificadas na pós-menopausa (Novella, Heras et al. 2012). Precocemente, níveis fisiológicos de TH podem melhorar ou reverter a disfunção endotelial. Em lesões ateroscleróticas avançadas, no entanto, a biologia celular existente propicia um substrato alterado e a iniciação tardia de TH aumenta a susceptibilidade a anormalidades inflamatórias e hemostáticas (Mendelsohn and Karas 2005). O mecanismo através do qual TH com estrogênio pode aumentar a ocorrência de eventos coronarianos em mulheres com placas ateroscleróticas complicadas não está completamente esclarecido, mas parece relacionar-se com a ativação e secreção de metaloproteinases (MMPs) (Finn, Nakano et al.2010).

Além disso, o próprio envelhecimento leva a um processo de diminuição na resposta vascular ao estrogênio. Essas modificações relacionam-se com metabolismo e níveis de estrogênio, número de receptores de estrogênio, distribuição, integridade e mecanismos de sinalização de receptores; bem como modificações estruturais na arquitetura vascular. Modificações induzidas pelo envelhecimento em outros hormônios sexuais tais como progesterona, podem influenciar os efeitos cardiovasculares dos estrogênios (Smiley and Khalil 2009) (figure 1).

Figure 1. Causas de diminuição da resposta ao estrogênio em decorrência do envelhecimento da vasculatura.



(Adaptado de Smiley and Khalil, 2009)

Outros fatores relacionados aos efeitos da TH sobre variáveis de risco CV e eventos CV na pós-menopausa parecem ser a dose e a via de administração utilizada. Maiores doses podem levar ao aumento de risco CV, em decorrência de distúrbios na trombogênese e remodelação vascular (Panay 2009). Menores doses de TH são associadas com menor risco de tromboembolismo, evento coronariano e acidente vascular cerebral, mas dados de ensaios clínicos randomizados que avaliem estes desfechos ainda não estão disponíveis .

A TH por via não oral não tem sido consistentemente associada à ativação de mecanismos inflamatórios ou pró-trombóticos (Olie, Canonico et al. 2011 ; Scarabin, Hemker et al 2011.). A TH não oral em baixas doses não demonstrou aumentar o risco de acidente vascular cerebral isquêmico (Renoux, Dell'aniello et al.2010). A utilização da via não oral evita o mecanismo de primeira passagem hepática e a ativação de citocinas pró-inflamatórias decorrentes do metabolismo hepático do estrogênio que ocorre após ingestão oral (Scarabin, Hemker et al.2011). No entanto, apresentaria um efeito menor sobre lipídeos (menor redução de LDL-c) e da circunferência da cintura, quando comparada a doses convencionais ou por via oral (Salpeter, Walsh et al. 2004).

Novos marcadores de risco cardiovascular como proteína C reativa e fibrinogênio, têm melhorado os modelos de avaliação de risco CV (Kaptoge, Di

Angelantonio et al.2013). Valores elevados de PCR são consistentemente associados com morte CV, acidente vascular cerebral isquêmico e morte por alguns tipos de câncer. No entanto, as associações entre PCR e estas desordens podem depender consideravelmente de fatores de risco CV convencionais e outros marcadores de inflamação, especialmente fibrinogênio (Kaptoge, Di Angelantonio et al.2010).

A família dos peptídeos natriuréticos tem sido considerada um importante sistema endócrino de origem cardiovascular e renal. Essas moléculas estão envolvidas em uma variedade de funções fisiológicas, incluindo vasodilatação, efeitos antiproliferativos, remodelação vascular e modulação do sistema renina-angiotensina-aldosterona. Este sistema é formado por 5 tipos de peptídeos natriuréticos: peptídeo natriurético atrial (ANP), que foi a primeira molécula a ser descoberta, em 1981; brain natriuretic peptide (BNP), C-natriuretic peptide (CNP), desdroaspis natriuretic peptide e urodilantin. Níveis de ANP no plasma estão aumentados em condições como falência cardíaca, infarto agudo do miocárdio, hipertrofia ventricular esquerda e hipertensão pulmonar(Chopra, Cherian et al. 2013). A medida de ANP tem demonstrado valor preditivo para prognóstico durante a ocorrência de doenças que afetam diretamente ou indiretamente a função cardíaca (Hayashi, Tsutamoto et al. 2001). Poucos estudam avaliam os efeitos da menopausa sobre ANP. Da mesma forma, os efeitos da TH sobre o sistema atrial natriurético ainda são pouco esclarecidos.

Em conclusão, a relação entre TH e fatores de risco CV é complexa e merece ser mais bem avaliada. Dose e tipo de estrogênio e progestogênio, via de administração, tempo de pós-menopausa, idade e a presença de fatores de risco CV parecem influenciar nos efeitos CV da TH.

Desenvolvemos ensaio clínico randomizado, crossover, com objetivo de avaliar os efeitos de dois tipos de TH (oral baixa dose e não oral) sobre proteína C reativa, peptídeo natriurético atrial e marcadores de risco CV tradicionais. Este estudo foi delineado considerando o conceito de “janela de oportunidade para TH”, ou “hipótese do tempo”. Selecionamos pacientes sintomáticas (com indicação de TH) e na pós-menopausa recente. Iniciamos os grupos de tratamento em janeiro de 2007 e completamos o tamanho de amostra esperado em 2011 (86 pacientes). Os dados referentes ao trabalho completo são aqui demonstrados (artigo original 1).

Realizamos também uma análise do TH não oral quanto aos efeitos da adição da progesterona natural micronizada ao estrogênio não oral (artigo original 2).

Durante o estudo, as pacientes utilizaram 2 tipos de TH não oral em doses equivalentes: 17 β estradiol 3 mg via nasal (n=40) ou 17 β estradiol 1.5 mg gel percutâneo (n=46), associados à progesterona natural micronizada 200 mg 14 dias por mês. Foi necessária a substituição do estradiol nasal por gel percutâneo por que o estradiol nasal parou de ser fabricado pelo laboratório responsável. Não houve diferença significativa, para todas as variáveis estudadas, quanto aos efeitos das 2 vias de TH não oral.

O terceiro artigo desta tese é uma revisão sistemática e meta-análise, onde sistematicamente buscamos todos os artigos que avaliassem os efeitos da TH baixa dose sobre peso, índice de massa corporal, pressão arterial, PCR e lipídeos em mulheres na pós-menopausa.

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Parte I

Low-dose oral or non-oral hormone therapy: effects on CRP and atrial natriuretic peptide in menopause (aceito para publicação: *Climateric*, 2014)

Low-dose oral or non-oral hormone therapy: effects on CRP and atrial natriuretic peptide in menopause

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Keywords: menopause, cardiovascular risk, hormone therapy, atrial natriuretic peptide, c-reactive protein, randomized clinical trial

ABSTRACT

Objective. To assess the effects of oral low-dose and non-oral hormone therapy (HT) on ultra-sensitive C reactive protein (CRP), atrial natriuretic peptide (ANP), and cardiovascular risk factors in postmenopause.

Methods. In this randomized crossover study, forty-four recent postmenopausal women, with no clinical evidence of cardiovascular disease, received oral low-dose HT (estradiol 1mg + drospirenone 2 mg/day) for 3 months. Forty-two patients received non-or al conventional HT (1.5 mg/day percutaneous 17β estradiol gel or equivalent for nasal route) for 3 months followed by 200 mg/day micronized progesterone by vaginal route (14 days during each menstrual period). After 3 months, patients were crossed-over without washout. Post-HT vs. pre-HT measures were determined: lipids, glucose, BMI, waist circumference, fibrinogen, CRP-stratified levels, ANP levels. The study was registered at clinical trials.gov (NCT01432028).

Results. Mean age was 51±3 years and the mean time since the menopause was 22±10 months. CRP- stratified high levels decreased in a higher number of non-oral HT patients, who moved to intermediate and low levels (P=0.02). No effect of HT was observed on ANP levels [baseline 67.4(18.4-104.5), low-dose oral 43.5(14.4-95.9), non-oral 39.8(15.5-67.5) pg/mL]. Markers of endothelial function did not worse with either low-dose oral or non-oral HT: von Willebrand factor (baseline 118±37%, low-dose oral 119±38%, non-oral 108±3%, p<0.01), fibrinogen (baseline 356±58 mg/dL; low-dose oral 343±77 mg/dL; non-oral 326±71 mg/dL, p<0,01).

Conclusions. Low-dose oral and non-oral HT for 6 months had neutral or beneficial effects in recent post-menopausal women with no clinical evidence of cardiovascular disease.

INTRODUCTION

The menopause is associated with changes in the hormonal and metabolic profiles. During the transition to the postmenopause, many women experience gains in weight and fat mass,[1] unfavorable changes in lipids (such as increased triglyceride and LDL-cholesterol levels and decreased HDL-cholesterol levels),[1] and increase in blood pressure and low-grade inflammation markers.[2] These changes may be due at least in part to the age-related impairment of physiological adaptation mechanisms; they are also possibly associated with estrogen deficiency[3,4] and the increase in cardiovascular (CV) risk that occurs in postmenopausal women.[5]

Hormone therapy (HT) is often indicated for treating menopausal symptoms and has been associated with reversal of some of these unfavorable changes. HT has been described to lower total and LDL-cholesterol[6,7] and to reduce waist circumference (WC).[8] However, clinical trials using conventional HT doses have reported adverse effects[7,9,10] and more prevalent CV events. Also, a few studies have shown that non-oral HT might have less adverse effects than oral preparations.[11] Non-oral HT avoids the hepatic first pass metabolism that is responsible for increasing liver proteins related to thrombosis and inflammation, such as C-reactive protein (CRP).[12]

These findings have raised the question of whether HT benefits could be maintained with lower doses[12,13] or alternative routes of administration.[12] Lower oral doses may be associated with lower induction of hepatic protein [12], but may also be associated with longer time to remission of symptoms of menopause[14]. In fact, few studies are available assessing the effect of an oral low-dose HT on CV risk factors

and symptoms of menopause in comparison to a non-oral conventional dose HT, even with no equivalent doses.

Cumulated data from clinical trials indicate that age and time since menopause can influence the cardiovascular response to HT.[15] In healthy recently postmenopausal women, HT has been associated with lower risk of coronary heart disease[16,17] and reduced overall mortality[16,18]. Emerging evidence suggests that the baseline clinical characteristics of women, including age, years since menopause and cardiovascular risk factors modify the individual's risk of coronary heart disease event even in the presence of HT.[9,19,20]

In that sense, biomarkers of CV risk, such as ultra-sensitive C reactive protein (CRP),[21] may provide important information regarding the possible interaction between HT and risk profile. Also the plasma levels of atrial natriuretic peptide (ANP) are increased in a variety of conditions, such as heart failure, acute myocardial infarction, hypertension, left ventricular hypertrophy and pulmonary hypertension. ANP levels increase with aging in both males and females,[22] and are associated with hyperestrogenic conditions.[23,24] Nevertheless, not much is known about the effects of estrogen on ANP and about the impact of HT on the atrial natriuretic system.

Therefore, the aim of the present study was to assess the effects of oral low-dose and non-oral HT on CRP, ANP and cardiovascular risk factors in a sample of recent postmenopausal women with no clinical evidence of cardiovascular disease.

METHODS

Patients

This randomized crossover study was carried out between March 2007 and April 2011, with women consulting for climacteric symptoms at the Gynecological Endocrinology Unit at a university hospital (Hospital de Clínicas de Porto Alegre) in Brazil. Non-hysterectomized, symptomatic, postmenopausal women fulfilling the following inclusion criteria were consecutively enrolled in the study: 1) last menstrual period between 6 months and 3 years before the beginning of the study plus follicle-stimulating hormone (FSH) levels higher than 35 IU/L; 2) age between 42 and 58 years; 3) no use of any medication known to interfere with hormonal, glucose or lipoprotein levels in the past 3 months; 4) no use of steroidal or nonsteroidal anti-inflammatory drugs in the last 15 days. Patients presenting diabetes, endometrial thickness higher than 0.5 cm, clinically relevant abnormal mammogram, history of cancer, thromboembolism or established cardiovascular disease were excluded.

The study protocol was approved by the Institutional Review Board and the local Ethics Committee, and written informed consent was obtained from every subject. The study was registered at clinical trials.gov (NCT01432028).

Study design and treatment

We consecutively enrolled 101 postmenopausal women fulfilling all the inclusion criteria. They were randomized into two groups of treatment. In order to generate a random sequence shuffled envelopes were used. Participants were allocated for first treatment through the sequentially numbered, opaque and sealed envelopes.

Some of the participants had been included in a previous study in which the same treatments were given for a shorter period of time.[25]

For the present study, the following protocol was used: the oral low-dose HT group (n=50 patients) received oral estradiol 1mg and drospirenone 2 mg/day (Angeliq®, Schering, Sao Paulo, Brazil) for three months. The non-oral conventional HT group (n=51 patients) received 1.5 mg/day 17β estradiol gel by percutaneous route (Oestrogel®, Farmoquímica, SP, Brazil) or the equivalent dose for the nasal route (Aerodiol®, Servier, RJ, Brazil) for three months; then, 200 mg/day micronized progesterone (Utrogestan®, Farmoquímica, Sao Paulo, Brazil) were added by the vaginal route, for 14 days during the three 28-day cycles. At the end of this first three-month period, the patients were crossed-over without washout for an additional 3 months. No differences were found in any of the studied variables among participants receiving the 17β estradiol by nasal or percutaneous route, as previously reported.[26]

Assessments

Clinical evaluation was performed before and monthly during the trial. Menopausal symptoms were graded using the Kupperman score.[27] Anthropometric measurements included body weight, height, waist circumference (measured at the midpoint between the lower rib margin and the iliac crest), hip circumference (measured at the level of the greater trochanter), waist-to-hip ratio (WHR), and body mass index (BMI, current measured weight in kg divided by height in m^2). Blood pressure was measured twice, at a 10-min interval in seated patients, using a digital sphygmomanometer (Omron HEM 742, Rio de Janeiro, Brazil) with appropriate cuff for the arm diameter.

Blood samples were collected before and at the end of three and six months of treatment. All samples were obtained between 08:00 and 10:00 a.m. after overnight fasting.

Total cholesterol, HDL-cholesterol, triglycerides, and glucose were determined by colorimetric-enzymatic methods using the Bayer 1650 Advia System (Mannheim, Germany). LDL cholesterol was estimated indirectly using the formula LDL = total cholesterol - HDL - triglycerides/5. Serum levels of luteinizing hormone (LH) and FSH were measured by electrochemiluminescence immunoassay (ECLIA), with intra and interassay coefficients of variation (CV) of 1.8% and 4.8%, respectively, for LH and 1.8% and 3.3% for FSH. The sensitivity of the assays was 0.12 IU/L for LH and 0.05 IU/L for FSH. Estradiol was measured by ECLIA (Roche Diagnostics, Mannheim, Germany), with an assay sensitivity of 5.0 pg/mL and intra and interassay CV of 5.7% and 6.4%. Serum insulin levels were measured using ECLIA (Roche Diagnostics, Mannheim, Germany), with sensitivity of 0.200 µIU/mL and intra and interassay CV of 2.0% and 4.3%, respectively. Fibrinogen was measured by the coagulometric method (Diagnostica Stago, Asnières, France), with sensitivity of 4 s and intra and interassay CV of 3.3% and 10.0%, respectively. CRP was assayed using stored specimens, with a validated high-sensitivity nephelometric method (Dade Behring Marburg, Marburg, Germany). Sensitivity was 0.17 mg/L and intra and interassay CV were 4.4% and 5.7%, respectively. For data analysis, individual results below the limit of sensitivity were considered as equal to 0.17 mg/L. Von Willebrand factor was measured by the immuno-turbidimetric assay (STA- Liatest, Diagnostica Stago, France). Sensitivity was 2% and intra and interassay CV were 1.9% and 2.9%, respectively. Circulating levels of ANP were measured in a sub-sample of 42 women before and during both treatments, using a

high-sensitivity EIA kit (EK-005-06; Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA). Blood samples for ANP analysis were placed in chilled tubes containing protease inhibitors (EDTA and PMSF, 10-5 mol/L; Pepstatin A, 0.5 x 10-5 mol/L; Sigma Co, St Louis, MO). To quantify plasma ANP levels, samples were extracted using Sep-Pak C-18 cartridges (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA) as previously described.[28] After drying in a Speed-Vac (Eppendorf 5301, Hamburg, Germany), the samples were dissolved in 500 ul phosphosaline buffer and the ANP levels were determined by EIA. All samples were measured simultaneously in duplicate. The intra-assay variation of the kit was <10%.

Statistical analysis

The sample size was estimated based on an interim analysis with the first 20 patients in each treatment group, considering a power of 80% and alpha of 5%. To detect a difference of 0.5 mg/L in CRP between baseline and post treatment, 80 women would be required.

Results are expressed as means±SD or median and interquartile range. Log10 transformation was used to normalize the distribution of non-Gaussian variables. Analysis of variance for Latin square design was used to assess time, treatment and carryover effects. No carryover effect was observed for any variable. Therefore, two-way analysis of variance (ANOVA) with repeated measures was carried out for comparing basal conditions, low oral and non-oral hormone therapy. Bonferroni adjustment was used for multiple comparisons. Changes in cardiovascular risk before and after HT, according to categorical CRP values, were estimated by the McNemar test. Spearman correlation coefficient was calculated to determine the relationship between ANP levels and blood pressure after HT.

All analyses were performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) and Stata (StataCorp LP, Texas, USA). Data were considered to be significant at $P<0.05$.

RESULTS

The mean age of participants was 51±3 years, and 96% were of Caucasian descent (the remaining 4% were of African descent). The age at menopause was 49.4±3 years, and the mean time since the menopause was 22±10 months. We randomized 101 women to treatment (Figure 1). Six patients in the low-dose oral HT group and nine patients in the conventional non-oral HT group dropped out in the first treatment round (Figure 1). Therefore, 86 patients completed the study. The baseline clinical characteristics were similar among groups in the first and second treatment rounds.

Table 1 shows Kupperman scores, anthropometric, hormonal and metabolic variables as well as markers of endothelial function and inflammation before and during treatment with low-dose oral HT or non-oral HT. At baseline, both groups were similar regarding all studied variables. Both treatments were equally effective in reducing symptoms and increasing serum estradiol levels. No changes were observed in BMI or weight, but waist circumference decreased significantly after both treatments. While total and LDL-cholesterol decreased significantly with both low oral and non-oral hormone therapy, HDL-cholesterol decreased only after low-dose oral treatment, and triglycerides decreased only after non-oral therapy. No effects were observed on fasting glucose or fasting insulin concentrations with either hormone treatment. CRP, fibrinogen and von Willebrand factor did not change after

low-dose oral HT. In the group receiving non-oral HT, fibrinogen and von Willebrand factor decreased after treatment, and C- reactive protein was unchanged.

When CRP levels were stratified according to low, intermediate or high levels (<1, 1 to 3 or >3 mg/L, respectively) no differences were found in levels of CRP in low-dose oral HT users. In contrast, fewer patients remained in the high levels of CRP after non-oral HT. These patients moved to the intermediate and low CRP levels (P=0.02) (Figure 2).

Table 2 shows ANP levels and blood pressure at baseline and after low-dose HT and non-oral HT. Blood pressure remained constant after HT. Non-significant reductions were observed in ANP levels after low-dose and non-oral HT. Table 3 shows the correlations between ANP levels and blood pressure. Non-significant correlations were observed.

DISCUSSION

In the present study, six months of treatment with low-dose oral or non-oral HT did not induce any deleterious effect on anthropometric, metabolic, and hormonal variables in recent and apparently healthy postmenopausal women. Also, no impact was observed on ANP or markers of inflammation or endothelial function. Moreover, non-oral HT was associated with a decrease in CRP-related CV risk, especially in women at the highest CV risk stratum according to CRP stratification. Very few studies have previously assessed the relationships between non-oral HT and ANP levels.

The present data confirm and expand our previous results obtained with shorter treatment duration and fewer participants. [25] Evidence suggests that the first months of HT may be critical because of the inflammatory activation and higher

prevalence of thromboembolic events.[29] Previous studies have reported significant changes in lipid profile and markers of endothelial dysfunction and inflammation[30] after 4 to 12 weeks of HT (30). However, some changes may not occur before 3 to 4 months of HT, as previously reported.[31]

HT has been shown to be more beneficial for women younger than 60 years of age and with less than 10 years of menopause onset.[32] Molecular mechanisms support the notion of a "window opportunity" for treating symptomatic postmenopausal women,[33] which seems to be related to pro-atherogenic and inflammatory changes in the CV system, intensified at and after the menopause.[34] Indeed, while in the early postmenopause HT may ameliorate or revert endothelial dysfunction, in the presence of advanced atherosclerotic lesions HT increases the susceptibility to hemostatic and inflammatory abnormalities.[35]

In addition, the presence of cardiometabolic risk factors at baseline increases CV risk when HT is given. [9,19,20] Therefore, measurements of lipids[20] and assessment of waist circumference, blood pressure, fasting glucose[19] and adiposity[1] may be clinically useful to identify women at increased risk for CV events during HT.[19] In the present study, participants had no previous cardiovascular disease and were in good general health. Consequently, weight, BMI, and fasting glucose remained unchanged during both low-dose oral and non-oral HT.

Interestingly, both treatments reduced waist circumference. In fact, menopause is more associated with increased central adiposity than with weight gain.[36-38] Evidence suggests that HT does not contribute to weight gain, and could even ameliorate accumulation of abdominal fat[38]. Few studies have assessed the influence of HT on waist circumference[25,39,40]. Taken together, the present results and these

previous reports support the idea that oral low-doses as well non-oral HT are associated with decreased WC in postmenopausal women[39,40].

Because non-oral HT does not have a hepatic first-pass effect seen after oral administration, its metabolic impact may differ from that of oral HT.[6] However, in the present study, we found amelioration of lipid profile, with no differences among the treatments. Non-oral HT promoted a decrease in total and LDL-C cholesterol and, in contrast to oral estrogens, caused a decrease in triglycerides. However, the changes were smaller than those induced with oral estrogens in equivalent doses.[6] In contrast, other studies in healthy women demonstrated a less powerful decrease in total (38) and LDL-C[41] with low-dose oral HT than with the conventional dosages.[13,42]

Fibrinogen,[43] CRP,[21] and von Willebrand factor (vWF) are regarded as surrogate markers of CV events and have been shown to be affected by HT.[9] Fibrinogen, a marker of acute phase and of destabilization of the atherosclerotic plaque,[43] is strongly associated with coronary heart disease risk.[9] Von Willebrand factor, a marker of the atherosclerotic process, is also associated with CV events[9]. Oral conventional doses has been associated with significantly increase of VWF[7]. In the present study, while no changes were found in fibrinogen and vWF after oral low-dose HT a decrease on both markers was found after non-oral HT. The present results confirm those from previous studies and seems to be related to the dose and route of administration of HT[44,45]. In this sense, Hemelaar et al (2005)[44] have also shown a reduction on the levels of VWF after non-oral HT, but no changes after oral low-dose.

CRP, a plasma protein synthesized by the liver, is a dynamic systemic marker of chronic inflammation. CRP concentration has continuous associations with the risk of coronary heart disease, ischemic stroke, vascular mortality, and death from several cancers,[21] but these associations may be dependent on other conventional CV risk

factors and fibrinogen concentration.[46] In fact, the real significance of higher or lower levels of CRP during HT on cardiovascular risk is still a debatable issue[9]. Regarding levels of CRP after HT, more women moved from the high levels of CRP to intermediate and low levels strata after non-oral HT. This is, at least in part, an effect of bypassing the first hepatic passage, because this mechanism prevents the synthesis of pro-inflammatory and pro-coagulant hepatic proteins, such as IL-6 and fibrinogen.[43] However, it is to note that using non-oral estradiol the CRP measured may also be coming from the vascular spaces.

Another important point related to HT concerns the influence of different progestin on clinical variables. Studies examining the effect of progestins on markers of inflammation have produced varying results [47]. Progestins with higher androgenic activity may interfere with lipid profile and glucose tolerance, and could affect the mechanisms of estrogen-induced CRP stimulation[48]. Recently, we have shown that the addition of micronized progesterone to estrogen did not worsen CRP, lipids, waist circumference or body mass index[26] , supporting the notion that micronized progesterone has a neutral effect on intermediate surrogate variables of cardiovascular risk[47].

We observed no differences between oral low-dose and non-oral HT in terms of ANP levels in this sample of postmenopausal healthy women. Conflicting results have been previously obtained when ANP levels were measured in postmenopausal women before and after HT. To the best of our knowledge, only three studies so far have evaluated the effects of HT on ANP in postmenopausal women. Maffei et al[49] found an increase in ANP and no changes in blood pressure after 3 months of non-oral HT (estradiol 50µg/day patches or gel 1mg/day; cyclically combined with oral 10 mg dihydrogesterone). Karjalainen et al[50] did not find any changes in ANP after 3 and

6 months of oral full dose HT (2mg/day estradiol) or non-oral full dose HT (1 mg/day 17βestradiol gel) in postmenopausal hysterectomized women. Spinetti et al,[51] studying a sample of recent post-menopausal women (up to 5 years since menopause; age range 46-53 years), reported a decrease on plasma ANP levels after oral HT (estradiol valerate 2 mg/day in combination with cyproterone acetate1 mg/day for 10 days) and non-oral HT (transdermal estradiol 17β 50μcg/day with medroxyprogesterone acetate 10 mg/day for 14 days). This suggests that the decrease in ANP reflects a restoration by HT of altered adaptive responses involved in blood pressure control by menopause.[22,52] We also found an absence of association between ANP and blood pressure as already reported by others.[53]

One limitation of the present study was the impossibility to perform double blinding for investigators and participants. However, our main outcomes are laboratorial determinations, which were performed by technicians who did not know what treatment had been used. In addition, analysis for carryover effects was performed and no carryover effect was found for any of studied variables.

In conclusion, both low-dose oral and non-oral HT for 6 months had similar neutral or beneficial effects on anthropometric and metabolic variables as well as on markers of inflammation and endothelial function in recent post-menopausal women with no clinical evidence of cardiovascular disease. No significant effects were found regarding ANP levels and HT. Further studies are needed in order to better understand the interaction between atrial natriuretic system and HT for menopause.

Conflicts of interest

The authors declare that they have no conflict of interest. The Authors alone are responsible for the content and writing of the paper.

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FIGURE LEGENDS

Figure 1. Flow of participants through the trial

Figure 2. CRP levels at baseline and after oral low-dose and non-oral hormone therapy (n=86). Low CRP levels: CRP <1 mg/L; intermediate CRP levels: CRP 1 to 3 mg/L; high CRP levels: CRP >3 mg/L. Baseline: before hormone therapy; oral: low-dose estradiol + drospirenone; nonoral: non-oral estradiol + micronized progesterone. *P=0.02 for differences between baseline and non-oral therapy and P=0.003 for differences between oral and non-oral therapy. McNemar test.

Table 1. Kupperman scores, hormonal, anthropometric, and metabolic variables and markers of inflammation and endothelial function at baseline and after oral low-dose and non-oral hormone therapy (n=86)

	Baseline	Oral	Non-oral	P
Kupperman index	26(17-30)[a]	3(0-6)[b]	4(0-8)[b]	<0.01
Estradiol (pg/mL)	13.1 (5-18.9)[a]	48.9(12.6-73.2)[b]	47.8(12.9-79.8)[b]	<0.01
Weight (Kg)	65.4±8.2	65.5±8.2	65.4±8.2	0.3
Waist circumference (cm)	84.2±7.3[a]	83±7.5[b]	83±7.3[b]	<0.01
Body mass index (kg/m^2)	26.2±3	26.1±3.1	26.2±3	0.5
Total cholesterol (mg/dL)	217.7±32.2[a]	199.4±35.3[b]	203.2±33.4[b]	<0.01
HDL-cholesterol (mg/dL)	63.3±15.7[a]	59.2±14.2[b]	60.7±15.7[a,b]	<0.01
LDL-cholesterol (mg/dL)	130.8±28.9[a]	117.2±29.5[b]	120.3±28.6[b]	<0.01
Triglycerides (mg/dL)	118.2±51.3[a,b]	125±57.3[a]	112±51.7[b]	0.02
Fasting glucose (mg/dL)	91.9±10	93±11	92.5±11.3	0.3
Insulin (µU/mL)	6.9(3.9-9.8)	7.2(4.2-10.2)	7(3.8-9.1)	0.1
C-reactive protein (mg/L)	1.51(0.36-2.8)	1.6(0.3-2.9)	1.19(0.3-2.1)	0.1
Von Willebrand factor (%)	118±37.5[a]	119.1±38[a]	108.7±33[b]	<0.01
Fibrinogen (mg/dL)	356.1±58.8[a]	343.5±77.9[a,b]	326.8±71.7[b]	<0.01

Baseline: before HT; oral: low-dose estradiol + drospirenone; nonoral: non-oral estradiol + micronized progesterone. Parametric variables: values expressed as means ± SD. Non-parametric variable: values expressed as median and interquartile range. P = two-way analysis of variance with repeated measures and adjustment with Bonferroni multiple-comparison correction (α<5%) . [a,b]Different superscript letters indicate statistical difference between correspondent groups. Ex. Kupperman index: [b]Oral and [b]Non-oral HT are significantly different from [a]Baseline but do not differ between each other.

Table 2. Atrial natriuretic peptide levels and blood pressure at baseline and after oral low-dose and non-oral hormone therapy (n=42)

	Basal	Oral	Non-oral	P
ANP (pg/mL)	67.4 (18.4-104.5)	43.5 (14.4-95.9)	39.8 (15.5-67.5)	0.7
SBP (mmHg)	118.7±13.5	116.2±12	116.7±12.1	0.2
DBP (mmHg)	76.2±7.2	76.6±8.2	74.9±9.7	0.2

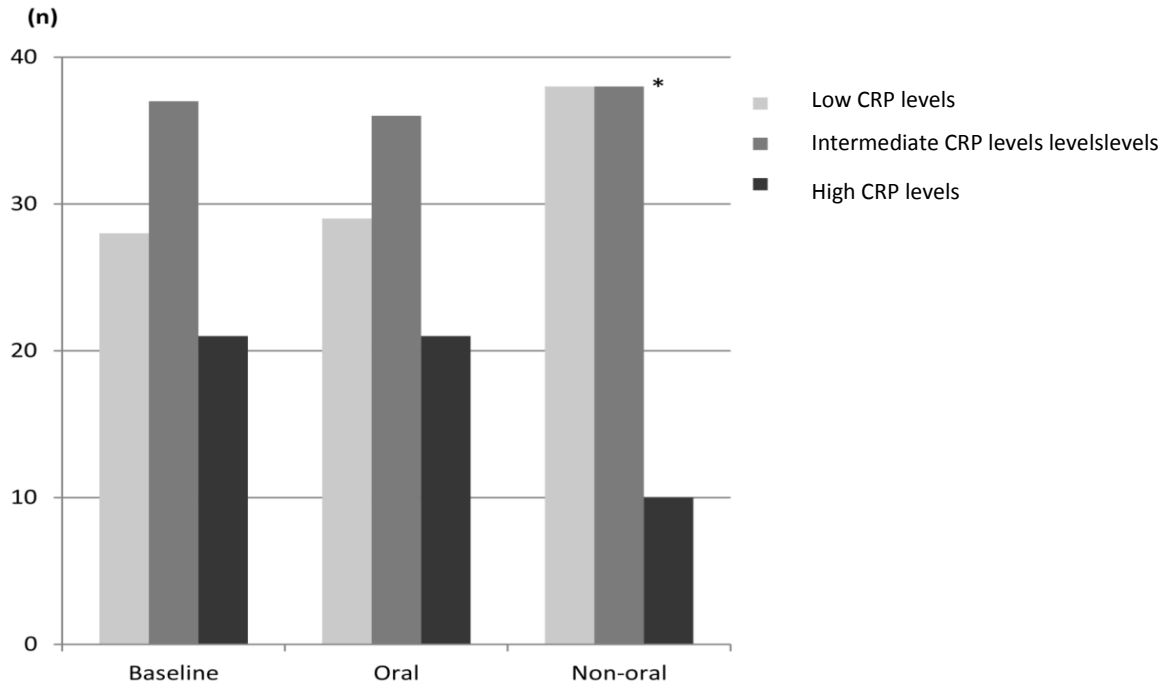
ANP: atrial natriuretic peptide; SBP: systolic blood pressure; DBP: diastolic blood pressure. Baseline: before HT; oral: low-dose estradiol + drospirenone; nonoral: non-oral estradiol + micronized progesterone. P = two-way analysis of variance with repeated measures. Parametric variables: values expressed as means ± SD. Non-parametric variable: values expressed as median and interquartile range.

Table 3. Correlations between levels of atrial natriuretic peptide and blood pressure (n=42)

	ANP levels (pg/mL)			
	Baseline		After HT	
	r	P	*r*	P
SBP (mmHg)	-0.06	0.6	0.2	0.1
DBP (mmHg)	0.2	0.08	-0.03	0.8

ANP: atrial natriuretic peptide; SBP: systolic blood pressure; DBP: diastolic blood pressure. Spearman correlation test.

Figure 2. CRP levels at baseline and after oral low-dose and non-oral hormone therapy (n=86).



Low CRP levels: CRP < 1 mg/L. Intermediate CRP levels: CRP 1 to 3 mg/L. High CRP levels: CRP > 3 mg/L. Baseline: before HT; oral: low-dose estradiol + drospirenone; nonoral: nonoral estradiol + micronized progesterone. * P = 0.02 for differences between baseline and non-oral HT and P= 0.003 for differences between oral and non-oral HT. McNemar test.

Supplementary Table. Baseline clinical characteristics of the participants according to first group of the HT (n=86)

	Oral	Non-oral	P
Kupperman index	25.7(22-30)	26(22-30)	0.7
Estradiol (pg/mL)	13.4(9-18.8)	12.1(8-20.5)	0.6
Age (years)	51.6±2.6	50.9±3.3	0.5
Age of menopause (years)	49.6±2.7	49.2±3.2	0.5
Waist circumference (cm)	83±6.5	85.3±8	0.1
Body mass index (kg/m^2)	26±3	26.5±3	0.8
SBP (mmHg)	121.5±11	116±14.5	0.1
DBP (mmHg)	77.1±6.2	75.3±8.2	0.2
Total cholesterol (mg/dL)	216.7±30.7	218±33.1	0.9
HDL-cholesterol (mg/dL)	65.3±16.5	61.2±14.9	0.2
LDL-cholesterol (mg/dL)	129.2±31.8	131.6±25.7	0.7
Triglycerides (mg/dL)	115.9±49	120.9±54	0.6
Fasting glucose (mg/dL)	92.1±11	91.6±9.2	0.8
C-reactive protein*(mg/L)	1.6(0.34-2.7)	1.4(0.41-2.76)	0.9
Von Willebrand factor (%)	113.6±33.8	121±40.3	0.3
Fibrinogen (mg/dL)	358.2±55.3	352.9±62.8	0.6

Oral: low-dose estradiol + drospirenone; nonoral: non-oral estradiol + micronized progesterone. SBP: systolic blood pressure. DBP: diastolic blood pressure. P = Independent samples T test. Parametric variables: values expressed as means ± SD. *Non-parametric variable: values expressed as median and interquartile range. (nonparametric variables were log converted for statistical analysis and reconverted for presentation in table format).

Parte II

Effects of micronized progesterone added to non-oral estradiol on lipids and cardiovascular risk factors in early menopause: a clinical trial (publicado em *Lipids in Health and Disease* 2012 11:133).

Effects of micronized progesterone added to non-oral estradiol on lipids and cardiovascular risk factors in early postmenopause: a clinical trial

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ABSTRACT

Background: Much attention has been drawn to the deleterious effects of adding progestins to estrogen as hormone therapy (HT) in postmenopausal women. Some widely prescribed progestins have been shown to partially oppose the beneficial effects of estrogens on surrogate markers of cardiovascular disease (CVD) risk. Progestins with higher androgenic activity may interfere with lipid profile and glucose tolerance, and could affect mechanisms of estrogen-induced C-reactive protein (CRP) stimulation. Recent data have shown that norpregnane derivatives, but not micronized progesterone, increase the risk of venous thromboembolism among transdermal estrogens users. The aim of the present study was to assess the effects of combining micronized progesterone with non-oral estrogen therapy on lipid profile and cardiovascular risk factors in a sample of early postmenopausal women. **Methods**: Clinical trial including 40 women receiving intranasal 17β estradiol 3 mg/day for two months and 46 women receiving percutaneous 17β estradiol gel 1.5 mg/day for three months (E2). Both groups received an additional 200 mg/day of micronized progesterone by vaginal route 14 days/month (E2+P). Outcome measures included body weight, waist circumference, body mass index (BMI), lipid profile and ultra-sensitive C-reactive protein (usCRP) at baseline and during the E2 or E2+P portions of treatment. **Results:** Mean age was 51±3 years. Mean time since menopause was 22.2±10 months. Most participants were overweight; HT did not change BMI. E2 and E2+P did not affect waist circumference and weight. Menopausal symptoms improved after HT. The effects of intranasal and percutaneous estradiol were similar, regardless of the addition of progesterone. Similarly, for the overall group of 86 women, micronized progesterone did not alter the response to E2. Blood pressure, glucose, insulin, HDL-c, triglycerides, and usCRP remained constant with or without micronized progesterone. Total cholesterol decreased after E2, and progesterone maintained this reduction. LDL-c levels were similar at baseline and with E2, and lower during E2+P in relation to baseline. **Conclusions:** Cyclic, short term exposure to vaginal micronized progesterone did not alter the metabolic and cardiovascular effects of non-oral E2 in early, apparently healthy, postmenopausal women.

Trial registration: ClinicalTrials.gov NCT01432028

Key words

Lipid profile; early postmenopause; micronized progesterone; non-oral estrogen; hormone therapy.

BACKGROUND

Much attention has been drawn to the deleterious effects of adding progestins to estrogen as hormone therapy (HT) in postmenopausal women [1] . Recent prospective randomized studies have raised great concern regarding this combination, which has been linked to a negative impact on the cardiovascular and venous systems and on cognition [2], as well as to the development of breast cancer [3] in women in the menopause transition and postmenopause.

Progestogens encompass both progesterone, the physiological molecule synthesized and secreted by the ovary, and synthetic compounds named progestins [4]. All progestins share a progestogenic effect that causes the endometrium to enter the secretory phase and determines a decrease in endometrial disease [5]. However, other biological effects of progestins vary widely, since each progestin or progestin metabolite binds to specific steroid receptors. Some of the most widely prescribed progestins have been shown to partially oppose the beneficial effects of estrogens on surrogate markers of cardiovascular disease (CVD) risk [4]. Progestins with higher androgenic activity may interfere with lipid profile and glucose tolerance [6], and could affect mechanisms of estrogen-induced C-reactive protein (CRP) stimulation [7].

Even though the route of estrogen administration is known to be an important determinant of cardiovascular risk in postmenopausal women using HT [8], recent data have shown that norpregnane derivatives, but not micronized progesterone, increase the risk of venous thromboembolism among transdermal estrogens users [9]. A few studies indicate that micronized progesterone may have a better risk profile with respect to variables related to cardiovascular risk [10, 11]. Therefore, not only the route of estrogen administration, but also the type of progestin may be important in determining the overall benefit-risk ratio for HT.

The aim of the present study was to assess the effects of combining natural micronized progesterone with non-oral estrogen therapy on variables related to lipid and hormonal profile and on ultra-sensitive C-reactive protein (usCRP) in a sample of early postmenopausal women.

METHODS

Study protocol

This study is nested within a crossover randomized trial assessing the comparative effects of low-dose oral HT and non-oral HT on cardiovascular risk factors and markers of endothelial function in early postmenopausal women. Preliminary results of this trial (which focused on the comparison between non-oral estradiol-micronized progesterone or low-dose oral estradiol-drospirenone therapy on metabolic variables and markers of endothelial function in early postmenopause), including the first 40 women enrolled, have been published [12].

In the present analysis, 42 women received 3 mg/day 17β estradiol by intranasal route (Aerodiol®, Servier, RJ, Brazil) for two months, and 53 received 1.5 mg/day 17β estradiol gel by percutaneous route (Oestrogel®, Farmoquímica, SP, Brazil) for three months. Additionally, both groups received 200 mg/day micronized progesterone (Utrogestan®, Farmoquímica, SP, Brazil) together with estrogen treatment by vaginal route 14 days/month during the studied cycles (during 2 or three months, respectively). The following were compared: effects of type of non-oral estradiol (intranasal or percutaneous gel) before, during the estradiol only portion of the study (E2) and during the estradiol plus progesterone portion (E2+P). The effects of E2 vs. E2+P for the overall group, regardless of route (intranasal or percutaneous), were also analyzed.

The 95 women enrolled for this trial fulfilled the following inclusion criteria: 1) last menstrual period between 6 months and 3 years before the beginning of the study plus follicle-stimulating hormone (FSH) levels higher than 35 IU/L; 2) age between 42 and 58 years; 3) no use of any medication known to interfere with hormonal, glucose or lipoprotein levels in the past 3 months; 4) no use of steroidal or non-steroidal anti-inflammatory drugs in the last 15 days. Patients presenting diabetes, previous hysterectomy, endometrial thickness higher than 0.5 cm, history of cancer, thromboembolism or established CVD were excluded.

Nine patients dropped out in the first two months of follow-up (Figure 1). Therefore, 86 patients completed the study. Clinical evaluation was performed before the treatment was begun and monthly during the trial. Anthropometric measurements included body weight, height, waist circumference (measured at the midpoint between the lower rib margin and the iliac crest), hip circumference (measured at the level of

the greater trochanter), waist-to-hip ratio (WHR), and BMI, current measured weight in kg divided by height in m^2). The Kupperman score was assessed before and during treatment [13]. Blood pressure was measured twice, at a 1-min interval in seated patients, using a digital sphygmomanometer (Omron HEM 742, Rio de Janeiro, Brazil) with appropriate cuff for the arm diameter [14].

The study protocol was approved by the Ethics Committee at the Hospital de Clínicas de Porto Alegre, and written informed consent was obtained from every subject (IRB 0000921/05-053). The study was registered at clinicaltrials.gov (NCT01432028).

Laboratory assessment

Blood samples were collected before treatment, during the estradiol-only portion (E2) (days 12 to 14 of the second treatment month), and at the end of treatment (days 24 to 28 of the last month of estradiol plus micronized progesterone administration) (E2+P). All samples were obtained between 08:00 and 10:00 a.m. After a 12-hour overnight fast, blood samples were drawn from an antecubital vein for determination of FSH, estradiol (E2), lipid profile (total cholesterol, HDL-c and triglycerides) plasma glucose (oral glucose tolerance test), and insulin. Blood samples were also drawn for us-CRP.

Total cholesterol, HDL-c, triglycerides, and glucose were determined by colorimetric-enzymatic methods using the Bayer 1650 Advia System (Mannheim, Germany). LDL-c was estimated indirectly using the formula LDL = total cholesterol - HDL-c - triglycerides / 5.

Serum FSH was measured by electrochemiluminescence immunoassay (ECLIA), with intra and interassay coefficients of variation (CV) of 1.8% and 3.3% for FSH. The sensitivity of the assay was 0.05 IU/L for FSH. Estradiol was measured by ECLIA (Roche Diagnostics, Mannheim, Germany), with an assay sensitivity of 5.0 pg/mL and intra and interassay CV of 5.7 and 6.4%. Serum insulin levels were measured using ECLIA (Roche Diagnostics, Mannheim, Germany), with sensitivity of 0.200 μIU/mL and intra and interassay CV of 2.0 and 4.3%, respectively. Ultra-sensitive CRP was assayed using stored specimens, with a validated high-sensitivity nephelometric method (Dade Behring Marburg, Marburg, Germany). Sensitivity was 0.17 mg/L and intra and interassay CV were 4.4 and 5.7%, respectively. For data

analysis, individual results below the limit of sensitivity were considered as equal to 0.17 mg/L.

Statistical analysis

Results are expressed as means ± standard deviation (SD) or median and interquartile range. Log10 transformation was used to normalize the distribution of non-Gaussian variables. Two-way analysis of variance (ANOVA) with repeated measures was carried out for comparing basal conditions, E2 and E2+P. Bonferroni adjustment was used for multiple comparisons. Friedman test was used for the analysis of Kupperman score, followed by the sign test. All analyses were performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) and Stata (StataCorp LP, Texas, USA). Data were considered to be significant at $P < 0.05$.

RESULTS

The mean age of participants was 51 ± 3 years, and 96% were Caucasian (the remaining 4% were of mixed African and European ancestry). Mean age at menopause was 49.4 ± 3 years, and mean time since menopause was 22.2 ± 10 months. Thirteen (15%) patients were smokers.

The effects of intranasal and percutaneous gel were similar during E2 and E2+P (Table 1). Table 2 presents body mass index (BMI), weight, waist circumference, blood pressure and Kupperman score for menopausal symptoms before and during E2 and E2+P in the overall group of 86 participants. Most participants were overweight. BMI did not change with HT. Similarly, waist circumference, weight and systolic and diastolic blood pressure remained unchanged during HT with E2 alone or E2+P. At baseline, all patients presented menopausal symptoms that improved significantly with treatment, as shown by the Kupperman score (Table 2).

Table 3 shows metabolic variables and usCRP at baseline and during treatment for the overall group. Glucose, insulin, high-density lipoprotein cholesterol (HDL-c), triglycerides, and the high-sensitivity C-reactive protein test (hsCRP) remained constant after non-oral therapy with or without micronized progesterone. Total cholesterol decreased after E2-only treatment, and the addition of progesterone maintained this reduction. Low-density lipoprotein cholesterol (LDL-c) levels were

similar at baseline and with E2 only, and were lower during E2+P treatment in relation to baseline.

DISCUSSION

In the present study, cyclic exposure to vaginally administered micronized progesterone over the short term failed to affect lipid profile in early and apparently healthy postmenopausal women. Several studies have evaluated the relationship between estrogen dose and/or route of administration and cardiovascular benefit-risk ratio of HT in postmenopausal women. More recently, observational studies began to draw attention to the impact of using specific types of progestin in combination with estrogen. However, there is a paucity of data derived from clinical trials to assess the effect of different progestogens on variables related to cardiovascular risk. Therefore, this work provides an important contribution toward clarifying the impact of combined micronized progesterone plus non-oral estrogen therapy.

Menopause is a risk factor for CVD because of the ensuing endogenous estrogen deficiency, which has a detrimental effect on cardiovascular function and metabolism. Even though there are biologically plausible mechanisms of cardiovascular protection against harm produced by estrogen therapy, recent clinical trials suggest that estrogen may be associated with cardiovascular risk rather than benefit in the postmenopause [12]. However, reanalysis of these studies has indicated a possible protective window in which recent postmenopausal women in their sixth decade may benefit from HT [1, 13]. In addition, it has been speculated that the cardioprotective benefits of HT may be more evident in the early postmenopausal period [14], although this is a controversial issue [15]. In the present study, the use of a sample of apparently healthy and relatively young (mean age of 51.3 ± 3 years) women who were postmenopausal for less than three years (22.2 ± 10 months) enabled us to more accurately demonstrate the neutral or beneficial effects of HT with both non-oral E2 alone or in combination with micronized progesterone- combined period.

Blood pressure levels remained unchanged after HT in the present sample. Previous studies have shown that non-oral estrogen therapy associated with micronized progesterone had no deleterious effects on blood pressure in normotensive and controlled hypertensive postmenopausal women [10, 16]. Estrogen increases the release of nitric oxide causing relaxation of smooth muscle cells and vasodilatation.

Progestins modulate the effects of estrogen on hepatic endocrine function through intrinsic androgenic properties. When co-administered with estrogen, progestogen may also have significant effects on body composition and metabolism because of its androgenic properties. The effects of HT on weight and body composition remain controversial [17]. In our study, non-oral E2 therapy with or without micronized progesterone did not modify waist circumference, BMI or body weight. Additionally, the treatment did not interfere with glucose or insulin levels, and reduced total cholesterol and LDL-c, supporting the notion that micronized progesterone has a neutral effect on intermediate surrogate variables of cardiovascular risk [18]. Dansuk et al. [19] evaluated the effects of five combinations of HT in postmenopausal women, including E2 alone and E2 associated with medroxyprogesterone (E2+MPA), noretisterone (E2+NETA), dydrogesterone (E2+DG) and micronized progesterone (E2+P). E2+NETA and E2+DG were found to improve insulin sensitivity after 3 months of treatment, whereas E2+P or E2 alone did not show such any effect in postmenopausal women.

It is well established that oral E2 therapy in conventional doses induces an increase in hsCRP, while transdermal E2 has either no effect on or even reduces hsCRP levels in postmenopausal women [20]. Studies examining the effect of progestins on markers of inflammation have produced varying results [18]. In the present study there was no worsening of hsCRP during HT with or without progesterone.

A limitation of this study is the short duration of treatment (6 months or less), since in clinical practice patients are usually treated for one year or more. Nevertheless, previous studies have reported significant changes in lipids and markers of endothelial function [21] after 4 to 12 weeks of HT. In addition, evidence suggests that there is a “critical period” in the first months of HT, related to greater inflammatory activation and higher thromboembolic risk events. Further studies of longer duration will be helpful to confirm our findings.

CONCLUSIONS

Data from the present study suggest that the addition of micronized progesterone to non-oral E2 did not induce harmful effects on variables related to cardiovascular risk in a population of healthy, early postmenopausal women. Micronized progesterone did not interfere with the effects of non-oral E2, and did not

abrogate the relief of symptoms. Finally, combined micronized progesterone and non-oral E2 treatment had neutral impact on blood pressure, body composition, lipid profile and markers of endothelial function.

List of abbreviations

ANOVA	Analysis of variance
BMI	Body mass index
CRP	C-reactive protein
CV	Coefficients of variation
CVD	Cardiovascular risk
DBP	Diastolic blood pressure
DG	Dydrogesterone
E2	Estradiol
ECLIA	Electrochemiluminescence immunoassay
FSH	Follicle-stimulating hormone
HDL-c	High-density lipoprotein cholesterol
hsCRP	High-sensitivity C-reactive protein test
HT	Hormone therapy
LDL-c	Low-density lipoprotein cholesterol
MPA	Medroxyprogesterone
NETA	Noretisterone
P	Progesterone
SBP	Systolic blood pressure
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
usCRP	Ultra-sensitive C-reactive protein
WC	Waist circumference
WHR	Waist-to-hip ratio

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1 - Representation of the design. Flowchart showing the total number of patients enrolled, number of dropouts, and group description.

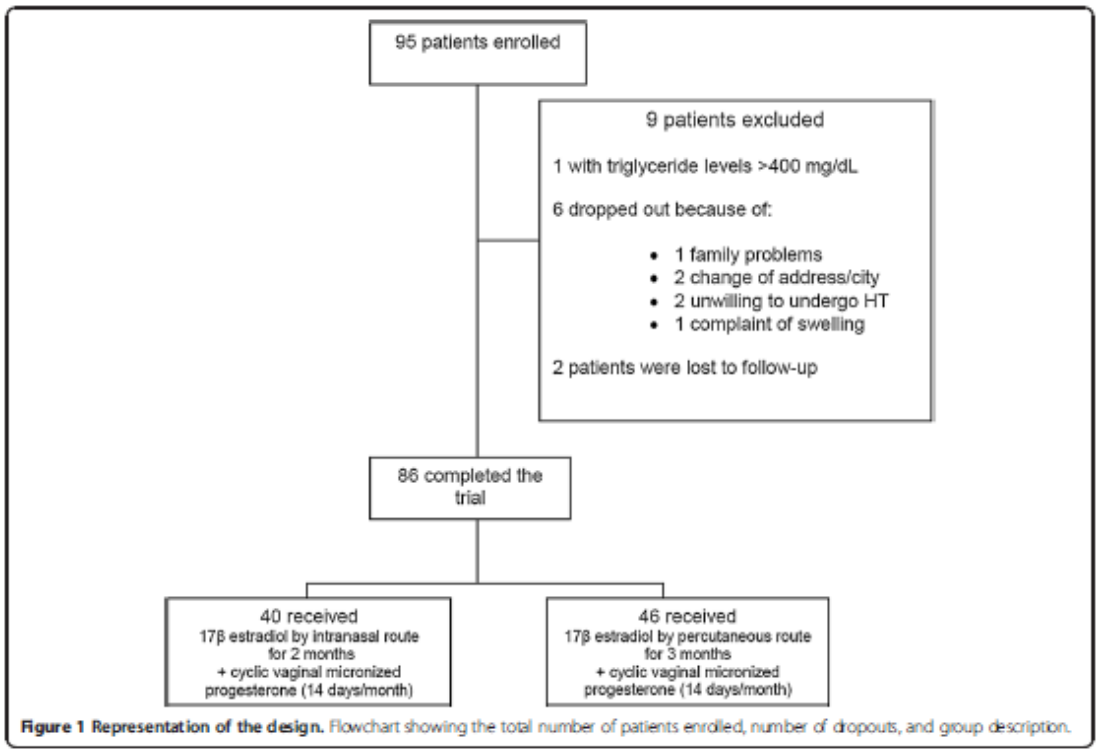

Figure 1 Representation of the design. Flowchart showing the total number of patients enrolled, number of dropouts, and group description.

Tables

Table 1 - Anthropometric and clinical variables according to type of non-oral estradiol (intranasal or percutaneous gel)

	Baseline		E2		E2+P		
	Intranasal n = 40	**Percutaneous n = 46**	**Intranasal n = 40**	**Percutaneous n = 46**	**Intranasal n = 40**	**Percutaneous n = 46**	***P***
BMI	26 ± 3	26 ± 3	26 ± 3	26 ± 3	26 ± 3	26 ± 3	0.6
Weight (kg)	66 ± 7	64 ± 9	66 ± 7	64 ± 9	66 ± 7	64 ± 10	0.6
WC (cm)	84 ± 6	84 ± 8	84 ± 6	84 ± 8	83.5 ± 5	83.6 ± 9	0.6
SBP (mmHg)	118 ± 15	119 ± 12	116 ± 15	114 ± 15	118 ± 14	116 ± 14	0.4
DBP (mmHg)	75 ± 7	77 ± 7	75 ± 9	74 ± 9	76 ± 10	74 ± 10	0.2
Total-c (mg/dL)	222 ± 31[a†]	211 ± 27[a†]	212 ± 31[b†]	201 ± 27[b†]	205 ± 31[b†]	200 ± 32[b†]	0.5
HDL-c (mg/dL)	63 ± 12	63 ± 18	62 ± 14	60 ± 15	63 ± 14	60 ± 16	0.2
LDL-c (mg/dL)	134 ± 28[a†]	125 ± 29[a†]	128 ± 32[a,b†]	118 ± 26[a,b†]	121 ± 26[b†]	117 ± 29[b†]	0.5
Triglycerides (mg/dL)	122 ± 49	117 ± 56	117 ± 48	114 ± 54	108 ± 42	115 ± 62	0.3
Fast glucose (mg/dL)	91 ± 11	92 ± 9	91 ± 8	91 ± 8	93 ± 11	91 ± 10	0.2

2h glucose (mg/dL)	106 ± 29	102 ± 23	113 ± 38	103 ± 35	110 ± 39	101 ± 31	0.2
Insulin (µU/mL)	7 (4-10)	6 (3-9)	6 (2-8)	7 (3-9)	6 (4-9)	7 (3-9)	0.2
Estradiol (pg/mL)	14 (8-17)	11 (5-20)	40 (9-121)	65 (20-119)	47 (13-68)	49 (10-96)	0.1
hsCRP (mg/L)	2.1 (0.4-3.9)	1.1 (0.3-2.3)	1.6 (0.5-3)	1.4 (0.2-3)	1.5 (0.5-2)	1 (0.2-2)	0.6

Values expressed as median and interquartile range or mean ± SD. Two-way analysis of variance with repeated measures (non parametric variables were log-converted for statistical analysis and reconverted for presentation in table format). P = difference between intranasal and percutaneous treatment.

† = $P < 0.01$ for difference between baseline, E2 and E2+P. Different superscript letters indicate statistical difference with Bonferroni multiple-comparison correction test ($\alpha < 5\%$).

Baseline: before hormone therapy; E2: estradiol only; E2+P estradiol + micronized progesterone.

Intranasal: 3 mg/day 17β estradiol by intranasal route (n = 40) for two months. Percutaneous: 1.5 mg/day 17β estradiol gel by percutaneous route (n = 53) for three months.

BMI: body mass index; DBP: diastolic blood pressure; HDL-c: high-density lipoprotein cholesterol; hsCRP: high-sensitivity C-reactive protein test; LDL-c: low-density lipoprotein cholesterol; SBP: systolic blood pressure; Total-c: total lipoprotein cholesterol; WC: waist circumference; 2h glucose: glucose levels 2 hours after 75g oral glucose load.

Table 2 - Clinical and anthropometric variables and menopausal symptoms (n = 86)

	Baseline	E2	E2+P	*P*
BMI	26.3 ± 3	26.29 ± 3	26.23 ± 3.1	0.8
Weight (kg)	65.5 ± 8.3	65.58 ± 8.3	65.4 ± 8.6	0.7
Waist circumference (cm)	84.3 ± 7.5	84.2 ± 7.6	83.5 ± 7.4	0.06
Systolic blood pressure	118.6 ± 13.5	115 ± 14.9	116.7 ± 14.3	0.1
Diastolic blood pressure	76.2 ± 7.3	74.7 ± 9.5	74.9 ± 9.7	0.4
Kupperman score	26 (17-30)[a]	6 (0-12.5)[b]	4 (0-8)[b]	< 0.01
E2 (pg/mL)	13 (5-19)[a]	54 (13-122)[b]	48 (13-80)[b]	< 0.01

Values expressed as median and interquartile range or mean ± SD. P = two-way analysis of variance with repeated measures (non parametric variables were log-converted for statistical analysis and reconverted for presentation in table format).

Different superscript letters indicate statistical difference with Bonferroni multiple-comparison correction test ($\alpha < 5\%$).

Baseline: before hormone therapy; E2: estradiol only; E2+P estradiol + micronized progesterone.

BMI: body mass index.

Table 3 - Anthropometric and metabolic variables and markers of endothelial function (n = 86)

	Baseline	E2	E2+P	*P*
Total-c (mg/dL)	216 ± 31[a]	207 ± 30[b]	203 ± 32[b]	< 0.01
HDL-c (mg/dL)	63 ± 16	61 ± 15	61 ± 15	0.06
LDL-c (mg/dL)	129 ± 29[a]	123 ± 29[a,b]	119 ± 28[b]	< 0.01
Triglycerides (mg/dL)	120 ± 53	115 ± 51	111 ± 53	0.2
Fast glucose (mg/dL)	91 ± 11	91 ± 8	92 ± 11	0.2
2h glucose (mg/dL)	103 ± 27	108 ± 36	105 ± 36	0.2
Insulin (µU/mL)	6.9 (3.9-9.7)	6.2 (2.8 -8.8)	6.9 (3.8 -9.1)	0.2
hsCRP (mg/L)	1.51 (0.38-2.9)	1.64 (0.4-3)	1.19 (0.3-2.1)	0.1

Values expressed as median and interquartile range or mean ± SD. P = two-way analysis of variance with repeated measures (non parametric variables were log-converted for statistical analysis and reconverted for presentation in table format).

Different superscript letters indicate statistical difference with Bonferroni multiple-comparison correction test ($\alpha < 5\%$)

Baseline: before hormone therapy; E2: estradiol only; E2+P estradiol + micronized progesterone.

HDL-c: high-density lipoprotein cholesterol; hsCRP: high-sensitivity C-reactive protein test; LDL-c: low-density lipoprotein cholesterol; Total-c: total lipoprotein cholesterol; 2h glucose: glucose levels 2 hours after 75g oral glucose load.

Parte III

Revisão sistemática e meta-análise

Effects of low-dose postmenopausal hormone therapy on variables related to cardiovascular risk: a systematic review and meta-analyses of randomized clinical trials

Effects of low-dose postmenopausal hormone therapy on variables related to cardiovascular risk: a systematic review and meta-analyses of randomized clinical trials

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ABSTRACT

In this systematic review and meta-analysis our objective was to evaluate the effects of low-dose estrogen HT on variables related to cardiovascular risk (blood pressure, weight, body mass index, c-reactive protein and lipids) in health postmenopausal women. MEDLINE (accessed by PubMed), Cochrane Central Register of Controlled Trials (Cochrane CENTRAL accessed by Wiley Science) and EMBASE databases were searched from 1990 through August 2013. We gathered data from randomized controlled trials that were designed to assess the effects of low-dose HT on variables related to cardiovascular risk in postmenopausal women. Two independent reviewers extracted data and assessed quality of the included studies. 28 trials involving 3360 patients were included in meta-analysis. A beneficial effect on body weight was noted: low-dose HT was associated with lower weight (-1.41kg, CI 95% -2.77,-0.05, P0.04). Low-dose HT showed that there was no deleterious effects on: BMI when compared to placebo (-0.09 kg/m², 95% CI -0.95,0.77) or conventional dose (0.45 kg/m², 95% CI -0.38,1.28); mean blood pressure when compared to placebo (-0.47mmHg, 95% CI -1.71,0.76) or conventional dose (0.26 mmHg, 95% CI -3.69, 4.20); C-reactive protein when compared to placebo (0.36 mg/L, 95% CI -0.14, 0.86) or conventional doses (-0.34 mg/L, CI 95% -0.94, 0.24). For lipids, low-dose HT was associated with lower levels of total cholesterol (-12.16 mg/dL, 95% CI -17.41, -6.92) and LDL-C (-12.16 mg/dL CI 95% -16.55, -7.77) when compared to placebo. Compared to conventional doses, lower doses were associated with high mean level of total cholesterol (5.05 mg/dL CI 95% 0.88, 9.21) and LDL-C (4.49 mg/dL, CI 95% 0.59, 8.39). The using of low-dose HT was not associated with differences in levels of triglycerides, when compared to placebo (-3.59 mg/dL, CI 95% -15.74, 8.55). Oral low-dose HT was associated with lower levels of triglycerides, when compared to conventional doses (-14.09 mg/dL, 95% CI -24.2, -3.93). For HDL-C, no significant differences were observed between users of low-dose HT, placebo or conventional doses. In conclusion, no deleterious effects were observed in postmenopausal health women after low-dose HT. The study was registered at PROSPERO. CRD 42013006520.

Keywords: Menopause. Hormone therapy. Low-dose. Cardiovascular risk. Meta-analysis.

INTRODUCTION

Estimates indicates that around 75% of women who are older than 50 years old will have hot flashes[1]. Hormone Therapy (HT) for menopause is the most efficient treatment for menopausal symptoms but their effects on CV risk remains controversial [2]. The publication of the Womens Health Innitiative study (WHI), in 2002, called the attention to a possible increase on the prevalence of cardiovascular (CV) events in postmenopausal women using HT [3,4]. The WHI study showed a significant increase in myocardial infarction, venous thromboembolism and stroke in postmenopausal women bearing hormone therapy (HT) *versus* those receiving placebo [4,5] and modified the existing knowledge, arising from observational studies [6] which showed a cardioprotective effect of HT in postmenopausal women. Detailed analysis of the WHI results highlighted factors that may be related to adverse events during therapy, such as aging[7], presence of CV risk factors [7, 8] and years of menopause [9]. In addition, it indicated the need for further clinical trials with lower doses and other routes of administration of HT, different from continuous conjugated equine estrogens (CEE) and medroxiprogesterone acetate (MPA) [10, 11] [12].

Conventional estrogen dose may produce supraphysiological plasma concentration in postmenopausal women, leading to estrogenic side-effects[13]. Additionally, conventional estrogen doses may be associated with CV harm due to disturbances in thrombogenesis and vascular remodelling[14]. Some studies have shown that lower HT doses have been related to lower risk of venous thromboembolism[15-17] and stroke[18,19]. However, randomized clinical trials showing comparative effects of conventional versus lower doses on the risk for CV events are not yet available[19]. Dose-dependent effects of HT on variables related to cardiovascular risk have been only shown in small clinical trials [10, 20-24]. In fact, the relationship between cardiovascular risk factors and the occurrence of cardiovascular events is complex, but women with the worse cardiovascular risk markers likely to be those with the highest risk of a CV event during HT [25, 26].

For a better understanding of the effect of low-dose HT on cardiovascular risk factors, data from these trials need to be evaluated to formulate a conclusion. Therefore, we conducted a systematic review and meta-analysis of pooled data from randomized clinical trials to evaluate the effects of low-dose HT on variables related to

cardiovascular risk (weight, body mass index, blood pressure, c-reactive protein and lipids) in postmenopausal women with no evidence of CV disease.

METHODS

This systematic review and meta-analyses was performed in accordance with the Cochrane Collaboration[27] and the Preferred Reporting Items for Systematic Reviews and Meta-Analysis: The PRISMA Statement[27].

Elegibility criteria and trial selection

We gathered data from randomized controlled trials that were designed to assess the effects of low dose HT on variables related to cardiovascular risk in postmenopausal women. Studies were included if they: (1) were randomized controlled trials of healthy postmenopausal women that compared low dose HT to placebo or HT conventional dose; (2) patients were at least 15 within each group of interest; (3) provided extractable data on at least one of these variables: blood pressure, body mass index, weight, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, C-reactive protein (CRP) and (4) the trials were published in English.

Low dose estrogen was considered as: 0.3 mg or less of conjugated equine estrogen, 1mg or less of oestradiol valerate or estradiol 17-β (oral); 100 μg/day or less of estradiol 17-β percutaneous gel, less than 50 μg/day of estradiol 17-β patches, less than 300 μg/day intranasal estradiol (non-oral) [28].

For studies with multiple low dose treatments, experimental group was chosen as the lowest dose of estrogen associated with the lowest dose of progesterone. The control group was defined as placebo or conventional doses of hormone therapy (HT). Studies with low dose estrogen, but without placebo or conventional doses for comparison were included in the discussion.

For studies with multiple publications from the same group of participants, the publication containing the most complete informations was chosen for inclusion. Regarding cross-over studies, two strategies were adopted: include the entire treatment time if the study clarifies the absence of carry-over effect in statistical analysis; or if the study does not describe this analysis, considering only the results of the first period of treatment.

Eligibility assessment was performed independently in an unblended standardized manner by 2 reviewers (GC and RBR) and any inconsistencies between these two reviewers were settled by the third reviewer (PMS) until a consensus was reached.

Search Strategy

The MEDLINE (accessed by PubMed), Cochrane Central Register of Controlled Trials (Cochrane CENTRAL accessed by Wiley Science) and EMBASE were searched comprehensively to identify randomized controlled trials published between January 1990 and August 2013, with low dose estrogen HT evaluating the effect of HT on blood pressure, body mass index, weight, lipids and C reactive protein.

Relevant trials were identified using the following procedure:

(1) Eletronic searches: We searched the MEDLINE, Cochrane Central Register of Controlled Trials and EMBASE eletronic databases for articles published between January 1990 and August 2013. The last search was run on August 2013. We used the following search terms to search all trials registers and databases: "Menopause"[Mesh]OR "Postmenopause"[Mesh] OR "Post-Menopause" OR "Post Menopause" OR "Post-Menopauses" OR "Postmenopausal Period" OR "Period, Postmenopausal" OR "Post-menopausal Period" OR "Period, Post-menopausal" OR "Post menopausal Period"; "Hormone Replacement Therapy"[Mesh] OR "Therapy, Hormone Replacement" OR "Hormone Replacement Therapies" OR "Replacement Therapies, Hormone" OR "Therapies, Hormone Replacement" OR "Replacement Therapy, Hormone"; (randomized controlled Trial [pt] OR controlled clinical trial [pt] OR randomized controlled trials [mh] OR random allocation [mh] OR double-blind method [mh] OR single-blind method [mh] or clinical trial [pt] OR clinical trials [mh] OR (“clinical trial’ [tw]) OR ((singl*[tw] OR doubl* [tw] OR trebl* [tw] OR tripl* [tw] AND (mask* [tw] OR blind* [tw])) OR (“latin square” [tw]) OR placebos [mh] OR placebo* [tw] OR random* [tw] OR research design [mh:noexp] OR comparative study [mh] OR evaluation studies [mh] OR follow-up studies [mh] OR prospective studies [mh] OR cross-over studies [mh] OR control* [tw] OR prospectiv* [tw] OR volunteer* [tw]) NOT (animal [mh] NOT human [mh]).

(2) Other sources: We searched ongoing randomized controlled trials, which had been registered as completed but not yet published, in http://ClinicalTrials.gov websites for information on registered randomized controlled trials. In addition, we searched the references of published studies and the relevant review and meta-analyses regarding the role of HT for postmenopausal women were examined for potential inclusive trials.

Data Extraction

Titles and abstracts of all articles identified by the search strategy were independently evaluated by two investigators (GC and RBR), in duplicate. Abstracts that did not provide sufficient information regarding the inclusion and exclusion criteria were selected for full-text evaluation. GC and RBR independently evaluated these full-text articles and made their selection in accordance with the eligibility criteria. Disagreements between reviewers were solved by consensus, and, if disagreement persisted, by a third reviewer (PMS). If the required data could not be located in published article, the author was contacted to provide the missing data of interest.

The data collected included: first author and study group name, publication year, periodic, number of patients, mean age, time of postmenopausal (when possible), pre-existing disease, medications, country, predominantly race, number of participants, intervention regimes, placebo or no treatment, duration off follow-up, values of the variables of interest and relevant references.

Assessment of risk of bias

We measured the quality of the trials included in this study as recommended by Cochrane Handbook for Systematic Reviews of Interventions (Higgins and Green, 2011), on the basis of adequate random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data and intention to treat analysis. Quality assessment was independently performed by two reviewers.

To assess publication bias, a funnel plot was created and analyzed by visual inspection.

Data analysis

Individual studies evaluated response variables before and after treatment in each arm. For this meta-analysis, pooled-effect estimates were obtained using the after treatment values since most studies did not provide enough information regarding before-after differences standard error (Higgins and Green, 2011). Results were presented as weighted means differences between treatment arms with 95% confidence intervals (CIs). Calculations were performed using a random effects model since we considered the individual studies not quite similar in characteristics in order to use fixed effect model. A P value < 0.05 was considered statistically significant. Heterogeneity was assessed using I^2 and Cochrane Q statistics. I^2 values greater than 25% were considered likely to indicate moderate heterogeneity and I^2 values above 50% indicative of substantial heterogeneity. All analyses were conducted using Review Manager software version 5.2 (Cochrane Collaboration).

Meta-analysis was performed by using two different analyses: 1) Low dose HT compared to placebo and 2) Low dose HT compared to conventional dose. Each analysis was considered a subgroup. Differences between subgroups were tested considering a P value < 0.05 as significative. Overall effect was demonstrate only when subgroups were not statistically significant.

For studies with multiple intervention groups (low dose and placebo and conventional dose) the following approach was performed to overcoming a unit-of-analysis error: the sample size of the low dose group (shared group) was divided by two generating two reasonably independent comparisons (low dose compared to placebo and low dose compared to conventional dose).

Sensitivity analysis were as it was pre-specified, including: route of administration (oral or non-oral estrogen HT) and type of HT (unopposed estrogen or combined (estrogen-progestin) HT). Non-oral low dose HT subgroup analysis was not done because of the scarcity of available studies (only two studies).

Regarding the blood pressure analyses, the mean arterial pressure was studied, calculated by the formula, PAM = 2/3 diastolic blood pressure + 1/3 systolic blood pressure [29].

RESULTS

Description of studies

The search strategy yielded 11.418 trials, from which 75 were considered as potentially relevant and were retrieved for detailed analysis. Twenty-eight trials met the

eligibility criteria and were included in the meta-analysis. The mean trial duration was 11.3 months (range 2-26 months) and the mean age of participants (±s.d) at baseline was 54.7±3.1 years.

Seven articles with low-dose TH were included in the discussion, but not entered in the meta-analysis. The reasons for this were: Six that evaluated only the low dose (no placebo or conventional dose for comparison) (Archer et al., 2005[30]; Endrikat et al., 2007[31]; Langer et al., 2006[32]; Lobo et al., 2000[33]; Pornel et al.,2002[34]; Yuksel et al., 2006[35]); and one (Ylikorkala et al., 2000[36]) not to provide the data to be appropriately placed in the meta-analysis. Two studies having incomplete data about one or more studied variables and not retrieved from the authors (Stevenson et al., 2005[23]; Tanko et al., 2005[37]).

Figure 1 shows flow diagram of the literature search and trials selection process. Table 1 summarizes the characteristics of meta-analyses included studies.

Table 1: Characteristics of included studies in our meta-analyses.

Study, year	N	Mean age, y	Intervention groups	Control	Follow-up (months)	Evaluated outcomes
Alexandersen et al., [38] 2001	301	57.9±3.8	1mg 17 β estradiol + 0.5mg NETA*; levormeloxifeno 1,25;5;10;20 mg	Placebo	13	Total cholesterol, HDL-C, LDL-C
Angerer et al., [39] 2001 (PHOREA)	197	58.3±4.5	1mg 17 β estradiol + 25 mcg gestodene 14d/m*; 1mg 17 β estradiol + 25 mcg gestodene 14days each three months	Placebo	12	BMI, Blood pressure
Bingol et al., [40] 2010	78	52.6±4.9	1mg 17 β estradiol + 0.5 NETA	Placebo	6	BMI, weight, blood pressure, CRP, total cholesterol, HDL-C, LDL-c, triglycerides
Brynhidsen et al., [41] 2004	266	55.2±4.8	0.025 mg 17 β estradiol TD + 0.125 mg NETA	Placebo	12	Total cholesterol, HDL-C, LDL-C, triglycerides
Casanova et al., [42] 2009	40	51±2.7	1mg 17 β estradiol + 2mg drospirenone; 300 mcg 17 β estradiol intranasal + 200mg micronized progesterone 14d/m		4	BMI, blood pressure, CRP, total cholesterol, HDL-C, LDL-C, triglycerides
Davidson et al., [43] 2000	264	58.1±5.8	1mg 17 β estradiol;1mg 17 β estradiol + 0.25 mg noretindrone acetate* ; 1mg 17 β estradiol + 0.5 mg noretindrone acetate	Placebo	6	Total cholesterol, HDL-C, LDL-C, triglycerides
Kraker et al., [44] 2004	362	55±5.1	1mg 17 β estradiol +5mg dydrogesterone;0.625 EEC+5mg AMP		13	Total cholesterol, HDL-C, LDL-C, triglycerides
Gambacciani et al., [45] 2011	70	52.7±0.5	1mg 17 β estradiol +2mg drospirenone	1000 mg cálcio	3	Blood pressure
Hemelaar et al., [46] 2003	152	54.4±4.3	50 mcg 17 β estradiol TD; 1mg 17 β estradiol; 1mg 17b estradiol+ 25 mcg gestodene*	Placebo	13	Total cholesterol, HDL-C, LDL-C, triglycerides
Hwang et al., [47]	222	60.8±6.6	1mg 17 β estradiol	Placebo	6	Total cholesterol, HDL-C, LDL-C, triglycerides

2005 (EPAT)						
Ichikawa et al.,[48] 2006	38	55.1±6.9	36mcg 17 β estradiol TD +2.5 mg AMP 12d/m; 0.625 mg CEE + 5 mg AMP 12d/m		12	Blood pressure
Kaya et al.,[49] 2007	80	50.8±3.5	1mg 17 β estradiol+10 mg dydrogesterone 14d/m	Placebo	12	Blood pressure
Kon Koh et al., [50] 2004	57	57±1	0.625 mg EEC+ 100 mg micronized progesterone; 0.3mg EEC + 100 mg micronized progesterone		2	CRP, total cholesterol, HDL-C, LDL-C, triglycerides
Lacut et al., [51] 2003	196	43-70	1mg 17β estradiol + 100 mg micronized progesterone; 50 mcg 17 β estradiol TD +100mg micronized progesterone 14 d/m	Placebo	6	CRP
Lobo et al., [10] 2001 (HOPE)	749	51.6±3.7	0.625 mg EEC;0.625 mg EEC+2.5 AMP; 0.45 mg EEC; 0.45 mg EEC+2.5 mg AMP; 0.45 mg EEC+1.5 mg AMP; 0.3mg EEC+1.5 mg AMP*; 0.3 EEC	Placebo	24	Total cholesterol, HDL-c, LDL-c, triglycerides
Loh et al., [20] 2002	96	53.9±7.6	1mg 17β estradiol+0.5 mg NETA; 2mg 17 β estradiol+1mg NETA		6	Total cholesterol, HDL-c, LDL-c
Odabasi et al., [52] 2007	120	50.5±2.7	1 mg 17 β estradiol + 0.5 mg NETA 2mg 17 β estradiol + 1 mg NETA		6	BMI, weight
Samsioe et al., [53] 2002	120	56	1mg 17β estradiol+0.25mg NETA* 1mg 17b estradiol+0.5 mg NETA	Placebo	12	Total cholesterol, HDL-C, LDL-C, triglycerides
Steiner et al., [54] 2005	222	61	1mg 17β estradiol	Placebo	24	Blood pressure
Stevenson et al., [23] 2005	579	56.4±4.7	1mg 17β estradiol+5mg dydrogesterone 14d/m*;1mg 17β estradiol+10 mg dydrogesterone 14d/m; 2mg 17β estradiol+10mg dydrogesterone 14d/m; 2mg 17β estradiol + 20mg diidrogesterone 14d/m	Placebo	26	HDL-C (Total cholesterol, LDL-C and tryglicerides included in systematic review)
Stork et al., [55] 2002 (PHOREA)	203	60.2±4.3	1mg of 17β estradiol+25 mcg gestodene 12d/m*; 1mg of 17β estradiol+25 mcg gestodene12d/m each three months	Placebo	12	CRP, total cholesterol, HDL-c, LDL-c, triglycerides
Tankó et al., [37] 2005	240	58±4	1mg 17β estradiol+1mg drosperinone*; 1mg 17β estradiol+2mg drosperinone; 1mg 17β estradiol+3mg drosperinone	Placebo	24	Blood pressure
Thorneycroft et al., [56] 2007 (HOPE)	822	51.6±3.7	0.625 mg CEE; 0.625 mg CEE+2.5 AMP; 0.45 mg CEE;0.45 mg CEE+2.5 mg AMP; 0.45 mg CEE+1.5 mg AMP; 0.3mg CEE+1.5 mg AMP*; 0.3 mg CEE	Placebo	24	Weight
Tugrul et al., [57] 2007	246	51.2±2.6	0.625 mg CEE + 2.5 AMP; 1mg 17β estradiol + 0.5 mg NETA		12	Weight, total cholesterol, HDL-c, LDL-c, triglycerides
Van Baal et al., [21] 1998	30	52±3	1mg 17β estradiol + 5 mg dydrogesterone*; 1mg 17β estradiol + 10 mg dydrogesterone	Placebo	15	CRP
Villa et al., [58] 2008	48	53.4±3.6	1mg 17β estradiol; 2mg 17β estradiol	Cálcio 500 mg	3	BMI
Villa et al., [59]	40	52±3.3	1mg of 17β estradiol + 2mg drospirenone	Placebo	6	Blood pressure, total cholesterol, HDL-c, LDL-

2011						c, triglycerides
Wakatsuki et al., [60] 2004	45	53.4±7.3	0.625 mg CEE; 0.3125 CCE	Placebo	3	CRP, total cholesterol, HDL-c, LDL-c, triglycerides

*Indicates the treatment at a low dose selected for meta-analyses (for studies with multiple low dose HT). TD= transdermal HT. CEE= conjugated equine estrogen. NETA= norethisterone acetate. AMP=medroxyprogesterone acetate. CRP= c-reactive protein. BMI=body mass index. d/m = days/month.

Risk of bias

The risk of bias summary of the twenty-eight studies including in meta-analysis is shown in the table 2. Funnel plots are show in appendix.

Quantitative Data Synthesis:

Effects of low dose HT

Weight

Data for the effect of low HT on weight was available from 4 trials totalizing 650 postmenopausal women. One of these studies evaluated low dose HT versus placebo (Bingol et al., 2010[40]). Two studies compared low dose HT with conventional HT dose (Odabasi et al., 2007[52]; Tugrul et al., 2007[57]). And one study (Thorneycroft et al., 2007[56]) had three arms. All studies included had as their treatment oestrogen-progestin.

Low dose HT when compared to placebo (-1.24 kg, 95% CI -3.15,0.67) or conventional dose (-1.26 kg, CI 95% -2.84,0.33) was associated with non-significant reduction on weight. The test for subgroup differences was non-significant (P=0.96). Overall, low dose HT was associated with lower weight (-1.41kg, CI 95% -2.77,-0.05, P0.04)(Fig.2).

Body Mass Index

Data for the effect of low dose HT on BMI was available from 5 trials, totalizing 375 postmenopausal women. Two studies evaluated low dose HT versus placebo (Angerer et al., 2001[39]; Bingol et al., 2010[40]). Two studies envolving low dose HT and conventional dose HT (Casanova et al., 2009[42]; Odabasi et al., 2007[52]) and one study(Villa et al., 2008[58]) had three arms. Only one study has been with unopposed estrogens (Villa et al., 2008). All studies including oral low-dose HT.

Low dose HT was not associated with changes on BMI, when compared to placebo (-0.09 kg/m², 95% CI -0.95,0.77) or conventional dose (0.45 kg/m², 95% CI -0.38,1.28). Overall, effects of low dose HT on BMI was not statistically significant (Fig.3). In sensitivity analysis of combined oestrogen-progestin low dose HT, no changes were identified in the results: low dose combined HT compared to placebo (-0.19, CI 95% -1.08, 0.69, p 0.67); low dose combined HT compared to conventional dose (0.46, CI 95% -0.39, 1.31, p 0.29) (data not shown).

Mean Blood Pressure

Data for the effect of low HT on mean blood pressure were available from 9 trials, wich included 843 postmenopausal women. Seven trials evaluated low dose HT versus placebo (Angerer et al., 2001[39]; Bingol et al., 2010[40]; Gambacciani et al., 2001[45]; Kaya et al., 2007[49]; Steiner et al., 2005[54]; Tanko et al., 2005[37]; Villa et al., 2011[59]). Two studies evaluated low-dose HT and conventional dose HT (Casanova et al., 2009[42]; Ichikawa et al., 2006[48]). Only one study have unopposed estrogens (Steiner et al., 2005).

Low dose HT was not associated with changes on MBP, when compared to placebo (-0.47mmHg, 95% CI -1.71,0.76) or conventional dose (0.26 mmHg, 95% CI -3.69, 4.20). Overall, low-dose HT did not reach statistical significant effect on PAM (Fig.4). Only one study have unopposed estrogens (Steiner et al., 2005). There were no substantial changes in the results when the analysis was perfomed without this study (-0.64 mmHg, 95% CI -2.03,0.76, data not shown).

C-Reactive Protein

Data for the effect of low HT on CRP were available from 7 trials, totalizing 614 postmenopausal women. Three studies evaluated low dose HT versus placebo (Bingol et al., 2010[40]; Stork et al., 2002[55]; Van Baal et al., 1998[21]). Two studies compared low dose HT with conventional dose HT (Casanova et al., 2009[42]; Koh et al., 2004[50]) and two studies (Wakatsuki et al., 2004[60], Lacut et al., 2003[51]) had three arms. Only one study has been with unopposed estrogen (Wakatsuki et al., 2004).

Low dose HT, when compared to placebo, was associated with non-significant differences on CRP (0.36 mg/L, CI 95% -0.14,0.86) (Fig.5). No changes were observed when compared low-dose HT to conventional dose (-0.35 mg/L, 95% CI -0.94, 0.24). Two studies contained transdermal agentes in the control group (Casanova et al., 2009,

Lacut et al., 2003). In the sensitivity analysis of low-dose oral HT compared to oral conventional HT a tendency to increase of CRP was observed with oral conventional doses (-0.67 mg/L, 91% CI -1.42,0.09, p 0.07, data not shown). In the results of combined oestrogen-progestin low-dose HT versus placebo, low-dose combined HT was not associated with substantial changes on CRP (- 0.11 mg/L, 95% CI -0.71, 0.49, $I^2$0%, data not shown).

Lipids

Data for the effect of low HT on lipids were available from 17 trials for total cholesterol (n=2321) and LDL-c (n=2323), 18 trials for HDL-c (n=2499) and 15 trials for triglycerides (n=2127). Nine studies evaluated to HT low dose versus placebo (Alexandersen et al., 2001[38]; Bingol et al., 2010[40]; Brynhildsen et al., 2004[41]; Davidson et al., 2000[43]; Hemelaar et al., 2003[46]; Hwang et al.,2005[47]; Lobo et al., 2001[10]; Samsioe et al.,2002[53]; Stork et al., 2002[55]; Villa et al., 2011[59]). Five studies compared to HT low dose with HT conventional dose (Casanova et al., 2009[42]; Koh et al., 2004[50]; Kraker et al., 2004[44]; Loh et al., 2002[20]; Tugrul et al., 2007[57]). Four studies have three arms (Lobo et al., 2001[10]; Stevenson et al., 2005[23] Villa et al., 2008[58]; Wakatsuki et al., 2004[60]).

Three studies were performed with unopposed estrogen (Hwang et al., 2005[47]; Villa et al., 2008[58]; Wakatsuki et al., 2004[60]). Two studies contained non-oral HT, in low dose group (Brynhildsen et al., 2004[41]) and in conventional dose group (Casanova et al., 2009[42]).

Total cholesterol

The group of patients of the low-dose HT had on average lower levels of total cholesterol, when compared to placebo (-12.16 mg/dL, CI 95% -17.41, - 6.92). On the other hand, in low-dose HT group was observed higher levels of total cholesterol (5.05 mg/dL, CI 95% 0.88, 9.21) (Fig.6).

In the sensitivity analysis of combined oestrogen-progestin HT, remained the similar results (oestrogen-progestin low-dose HT compared to placebo -12.21 mg/dL, CI 95% -17.83, -6.60, p <0.001; oestrogen-progestin low-dose HT compared to conventional dose 5.1 mg/dL, CI 95% 0.96, 9.42, p 0.02) (data not shown). For sensitivity analysis of oral low-dose agents was observed the same effect on TC (compared to placebo -11.30 mg/dL, 95% CI -16.94, -5.67, p < 0.001; compared to

conventional dose 5.4 mg/dL, 95% CI 1.10, 9.83, p 0.02) (data not shown). Significant heterogeneity was verified for total cholesterol compared to placebo. Sensitivity analyses did not reduced heterogeneity in this subgroup.

LDL-C

The low-dose HT group, when compared to placebo group, had on average lower LDL-c (-c (-12.16 mg/dL, CI 95% -16.55, - 7.77). Conversely, significant increase in LDL-c was observed in low-dose group when compared to conventional dose group (4.49 mg/dL, CI 95% 0.59, 8.39) (Fig.7). Oestrogen-progestin low-dose HT sensitivity analysis was not associated with substantial changes in the results (oestrogen-progestin low-dose HT compared to placebo -11.8 mg/dL, CI 95% -15.57, -8.1, p <0.001; oestrogen-progestin low-dose HT compared to conventional dose 4.4 mg/dL, CI 95% 0.45, 8.37, p 0.03) (data not shown). Oral low-dose agents also produced similar results (compared to placebo -11.6 mg/dL, CI 95% -15.5, -7.6, p <0.001; compared to conventional dose 5.18 mg/dL, CI 95% 1.04, 9.3, p < 0.001)(data not shown).

HDL-C

Low-dose HT showed no significant effects on HDL-c compared to placebo (1.42 mg/dL, CI 95% -2.75, 5.58) or conventional dose (1.01 mg/dL, CI 95% -2.32, 4.33)(Fig.8). Oestrogen-progestin low-dose HT sensitivity analysis was not associated with substantial differences on HDL-c (compared to placebo 1.64 mg/dL, CI 95% -3.4, 6.7; compared to conventional dose 1.2 mg/dL, CI 95% -2.6, 5.0) (data not shown). For oral low-dose HT agents, was observed very similar results (compared to placebo 1.4 mg/dL, CI 95% -3.2, 6.0; compared to conventional dose 1.4 mg/dL, CI 95% -2.2, 5.0). Sensitivity analyzes did not explain the heterogeneity observed.

For HDL-C, because of high heterogeneity, additional analyses were conduzed. Removing this studies with more androgenic progestins (Lobo et al., 2001[10];Loh et al., 2002[20]; Tugrul et al., 2007[57]) in the subgroup analyses low-dose compared to conventional dose, the heterogeneity reduced for 0% with no change in the effects on HDL-C (0.25 mg/dL, CI 95% -1.89, 2.39, I^2 0%, data not shown).

Triglycerides

No significant effects were observed when compared low-dose HT to placebo (-3.59 mg/dL, CI 95% -15.74, 8.55) or conventional doses (-6.75 mg/dL, CI 95% -14.9, 1.4) (Fig.9). Significant heterogeneity was observed in both subgroups. In sensitivity analyses of oral agents, was observed significantly lower levels of triglycerides in low-dose group when compared to conventional dose group (-14.09 mg/dL, CI 95% -24.2, -3.93) ($p<0.01$) (data not shown). For oral low-dose HT compared to placebo, no significant differences were observed (1.01 mg/dL, CI 95% -13.5, 11.5) (data not shown).

Removing two unopposed estrogen studies (Villa et al., 2008; Wakatsuki et al., 2004) result in decrease heterogeneity and significant lower levels of triglycerides when oral low-dose HT was compared to oral conventional dose (-11.1 mg/dL, CI 95% -17.1, -5.02, $I^2$0%) (data not shown).

DISCUSSION

In this systematic review and meta-analysis of low-dose HT RCTs, 28 clinical trials were pooled with postmenopausal women without overt clinical disease, including a total of 3360 participants, with a mean age of 54.7 years and mean follow-up of 11.3 months. In this population of apparently healthy postmenopausal women, low-dose HT did not induce deleterious effects on mean blood pressure, BMI, total cholesterol, HDL-c, LDL-c, triglycerides and a beneficial effect on body weight was found.

Menopause is associated with changes in body composition and weight gain [61-63]. Studies in ovariectomized mice suggest that decreased levels of estrogen after ovariectomy determines a rapid weight gain, reversed after HT[64]. Evidence suggests that the same occur with postmenopausal women. Regarding low-dose HT our review has obtained only four studies assessing the effects of this treatment on body weight but the results are consistent with a significant reduction on weight after treatment. In contrast, Springer ET AL (2013)[65], looking for the effects of HT on body weight and its association with leptin levels, found that current literature does not compile evidence for an effect of HT in attenuating the weight gain observed in postmenopause. One difference between the Springer review and present data is that we stratified the RCTs regarding HT by the different doses and, thus, we could observe that the effect of reducing or maintaining body weight may be related to the dose of HT.

In this meta-analysis, low-dose HT was associated with maintenance of BMI. Gravena et al (2013)[66], in a population-based study of 456 Brazilian postmenopausal women, found that one of the factors most strongly associated with overweight at this stage of life was not using HT. A review that aimed to summarize the literature regarding the impact of the menopause on body weight and body composition concluded that estrogen-only or estrogen-progestin therapy does not adversely affect body weight and may ameliorate accumulation of abdomninal fat [67]. Reduction of central fat accumulation was also observed in clinical trials with low-dose HT [35]. The North American Menopause Society states that hormonal therapy, regardless of the type (estrogen or estrogenprogestogen), does not cause overweight [2]. Obesity is a major factor for diabetes mellitus and cardiovascular diseases, and several factors may be related to obesity in postmenopausal women, such as level of physical activity, parity, level of education and history of obesity [66, 67] and HT[62]. Further studies are needed, ideally through randomized clinical trials, for better evaluate the complex relationship between low dose HT, and changes in body composition and weight gain, as well as to identify which women can benefit from the favorable effects of HT on body composition.

Data regarding the effects of HT on blood pressure in postmenopausal women with and without hypertension is controversial. In our meta-analysis, we found 9 trials with low-dose HT in not hypertensive women, and low-dose HT was not associated with changes in mean arterial pressure. Four studies [37, 42, 45, 59] reporting the effects of 1mg estradiol associated with drospirenone, in non-hypertensive women, showed neutral effect on blood pressure. Angerer et al (2001)[39] and Bingol et al (2010)[40], studying oral estradiol 1mg associated with gestodene and NETA, respectively, found a reduction in diastolic BP, while systolic BP remained unchanged. In contrast, the WHI study [4] found a significant rise in systolic blood pressure in women on HT versus placebo after 2 years of therapy. The PEPI trial [68] also found an increase in systolic blood pressure in all HT groups after 3 years. Differences among trials may be attributable to several factors. Both WHI and PEPI trials used conventional doses of HT and conjugated equine estrogens. In addition, progestogen regimens also differed.

In the present meta-analysis, in which all compiled studies used the oral low-dose, CRP remained unchanged after low-dose HT. We also found a trend toward higher CRP when comparing full dose HT with low-dose oral HT, although this result should be regarded with caution because of the small number of studies. The increase in CRP levels after oral HT has been consistently observed [8, 68, 69] after oral agents in conventional doses [70] and a study that compared the effects of oral and non-oral HT on CRP reported significantly differences [70]. In fact, current literature data indicates a neutral profile of non-oral HT on CRP [71, 72]. Although CRP levels may be regarded as a predictor of coronary heart disease, mortality and stroke [73], the relationship between the increased of CRP in postmenopausal women after HT and cardiovascular events is complex and unclear.

In this meta-analysis, a significant reduction in total cholesterol and LDL-C was observed in the low dose compared to placebo. When low dose was compared to full dose, the full dose was more effective in reducing TC and LDL-C. These results confirm already published data on the effects of HT on lipids[34, 70]. In the present review we observed a reduction on triglycerides when using low-dose oral of HT. Dose dependent effect of oral HT on TGL have been reported [11, 74], as well as different actions of oral and non-oral HT on triglycerides[70, 75]. Transdermal-administered estrogen has lesser effect on serum lipids, probably due to the by-pass of portal circulation and thus, minimal effect on hepatic metabolism[76]. This meta-analysis was not able to assess differences between oral and non-oral low doses HT with respect to effects on lipids, because only one study was available analyzing low-dose non-oral HT[41].

We observed significant heterogeneity in the analyses of HDL-C. This heterogeneity limits the interpretation of the data and points to the absence of effects of low-dose HT on HDL-C. Same studies discuss whether different types and regimens (ciclic or continuous) of progestins can influence on HDL-c levels [30, 32, 71, 76]. More androgenic progestins may have an action on the reduction of HDL-c [31]. Using sensitivity analyzes in order to explain the heterogeneity showed that removing studies with more androgenic progestins[11, 20, 57] resulted in heterogeneity (I^2) 0% and unchanged HDL-c levels. These findings lead to other intriguing questions about the

relationship between oestrogen doses and cyclic or continuous progestin regimens[15, 76, 77].

Limitations of the present meta-analysis are as follows: 1) Reduced number of studies for some variables; 2) Different doses, regimens and molecules used for and HT; 3) diverse times of follow-up among the pooled studies, that could have affected our results, and 4) choosing the English literature studies. These limitations highlight the need for further clinical trials in order to clarify the observed interactions between doses of HT and systemic effects.

In conclusion, the present results support the notion that low-dose HT had no harmful effects on lipids and mean blood pressure and possibly have a beneficial effect on weight in apparently healthy postmenopausal women. Further studies are needed for proper understanding of the mechanisms involved on the effects of low-doses HT by the oral route on CRP.

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Figure 1. Flow diagram of the literature search and trials selection process.

Figure 2. Effects of low-dose HT on weight.

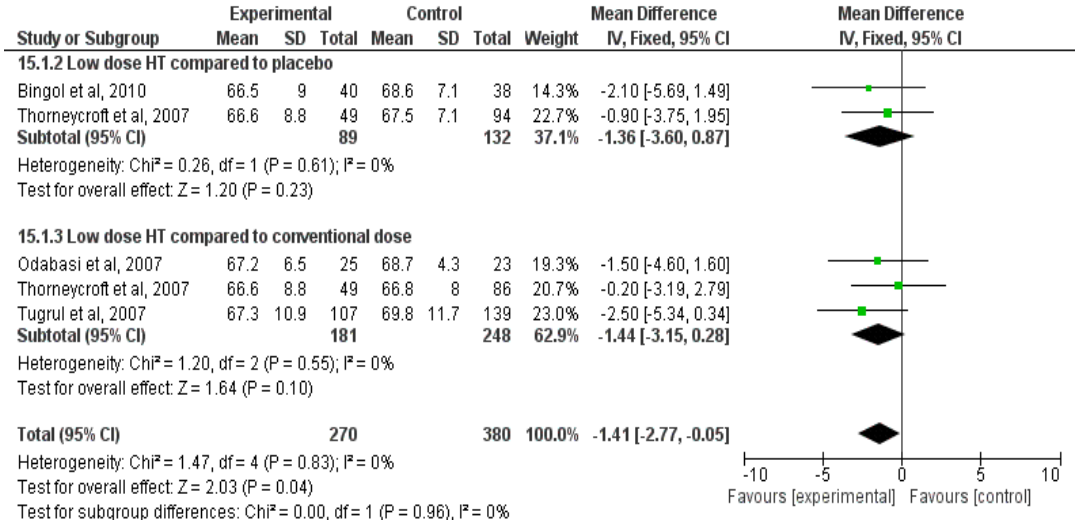


Figure 3. Effects of low-dose HT on body mass index.

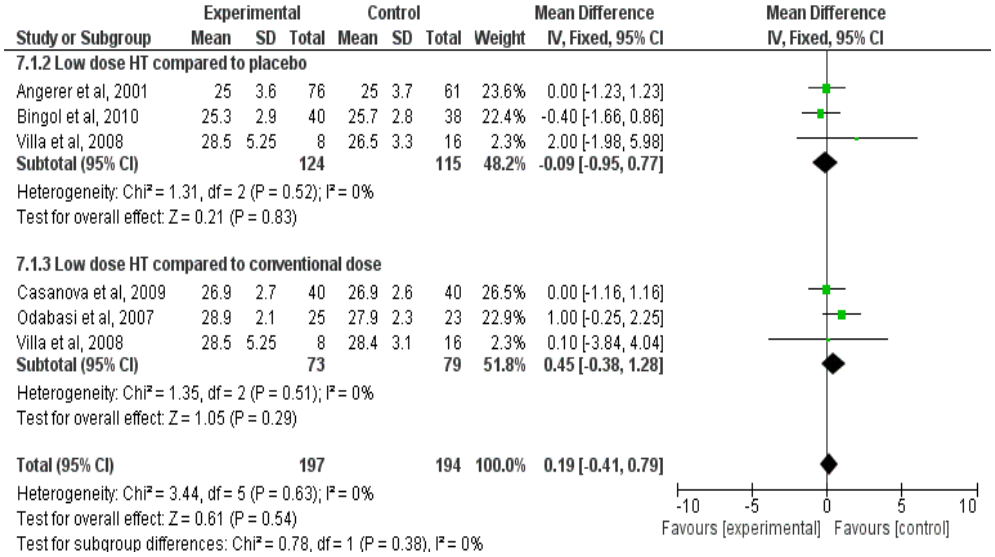


Figure 4. Effects of low-dose HT on mean blood pressure.

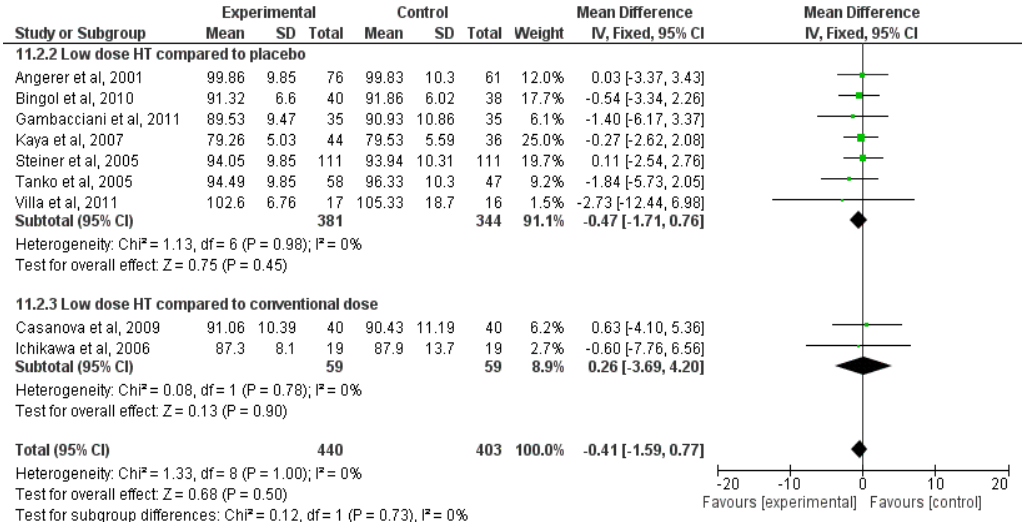


Figure 5. Effects of low-dose HT on C-reactive protein.

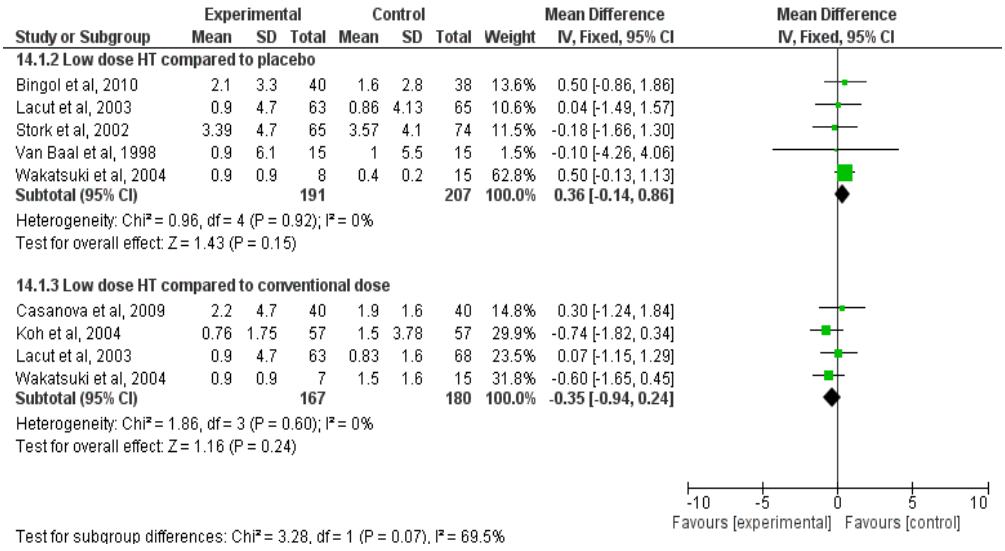
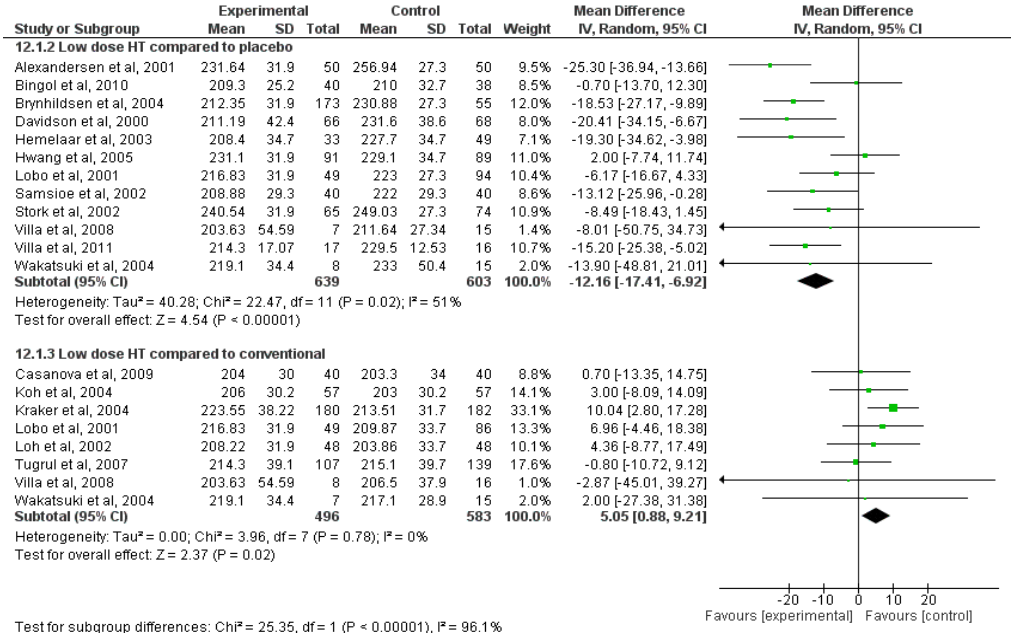


Figure 6. Effects of low-dose HT on total cholesterol.



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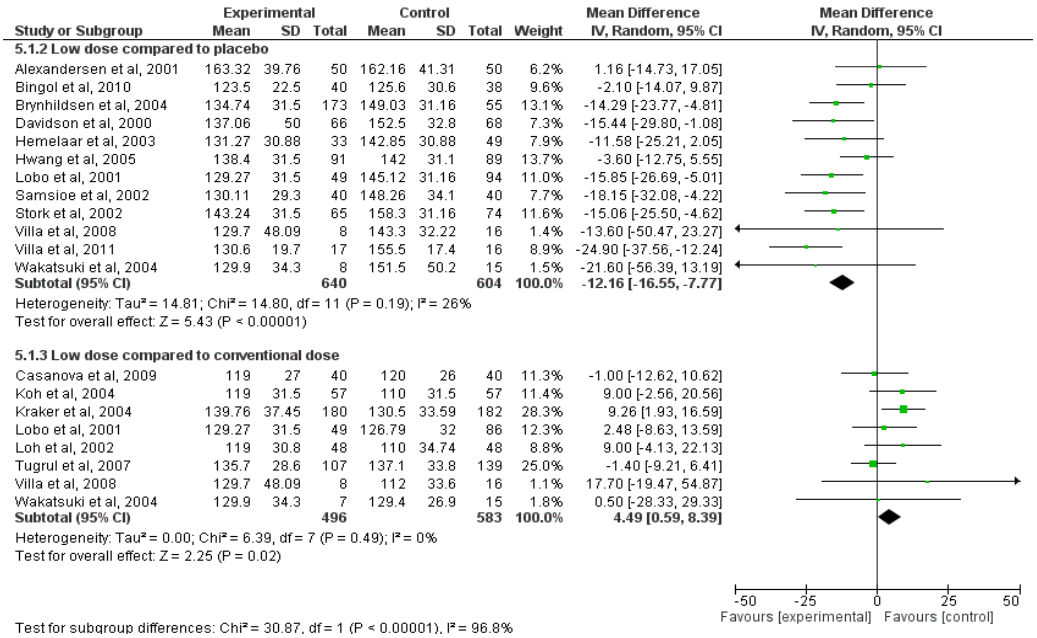
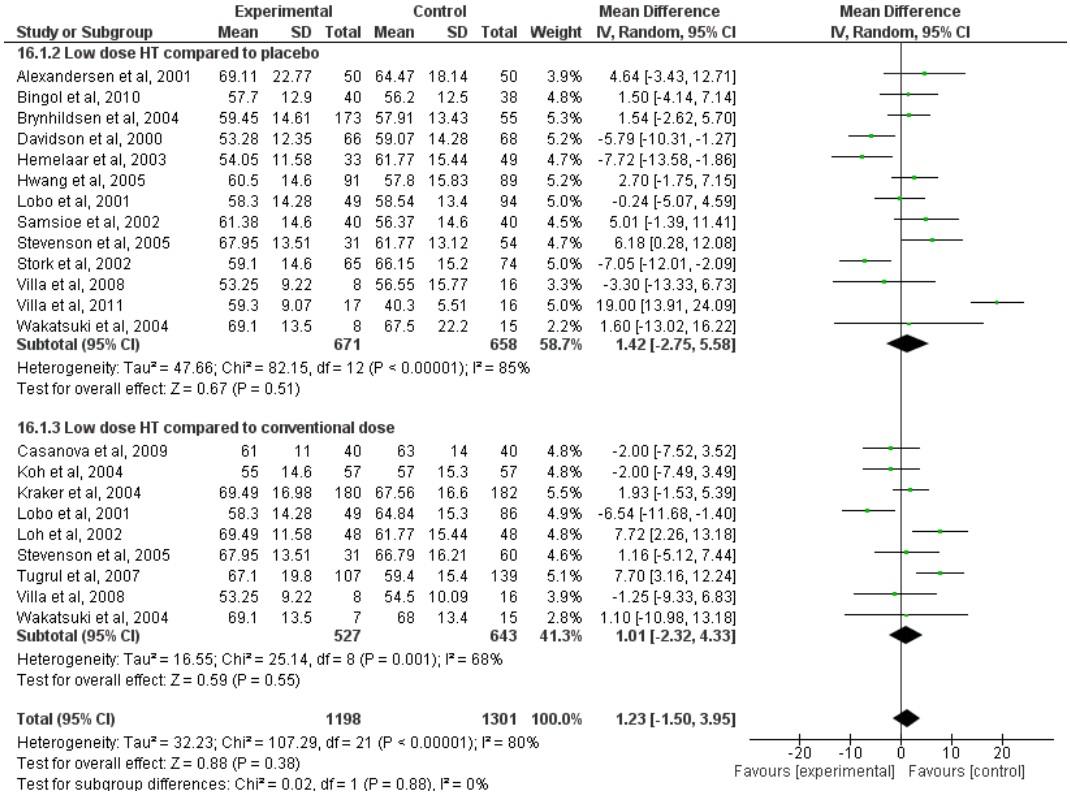


Figure 8. Effects of low-dose HT on HDL-cholesterol.



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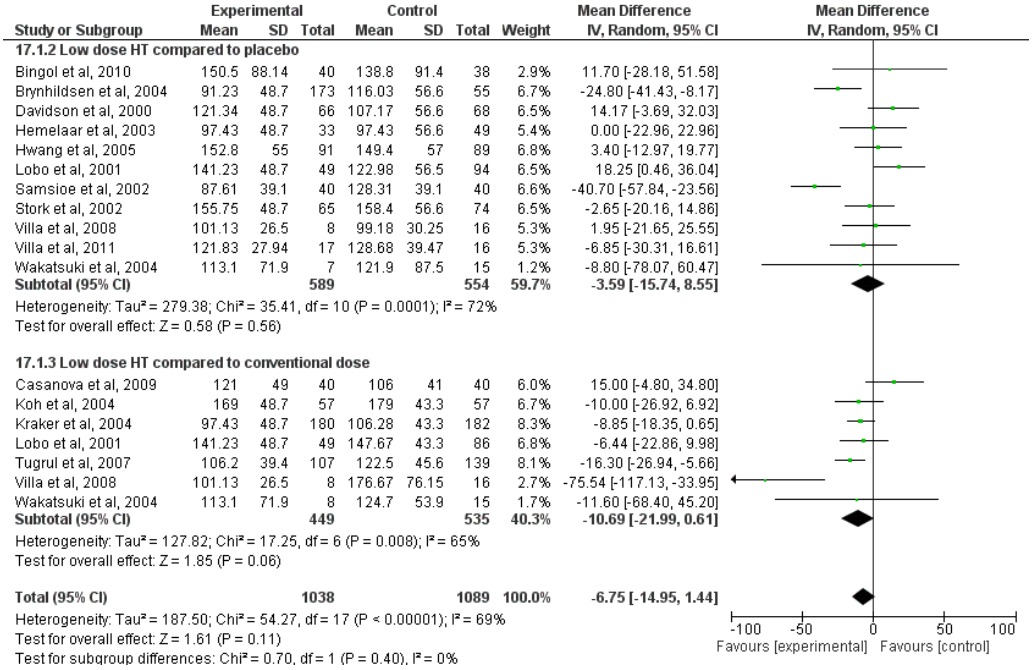
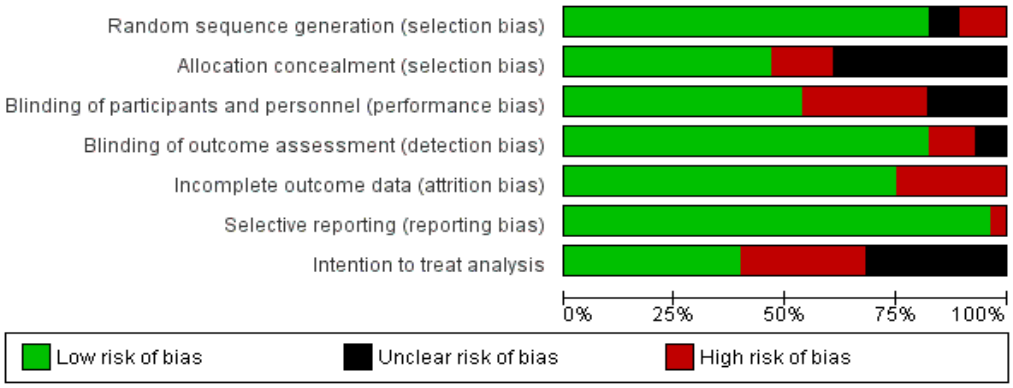


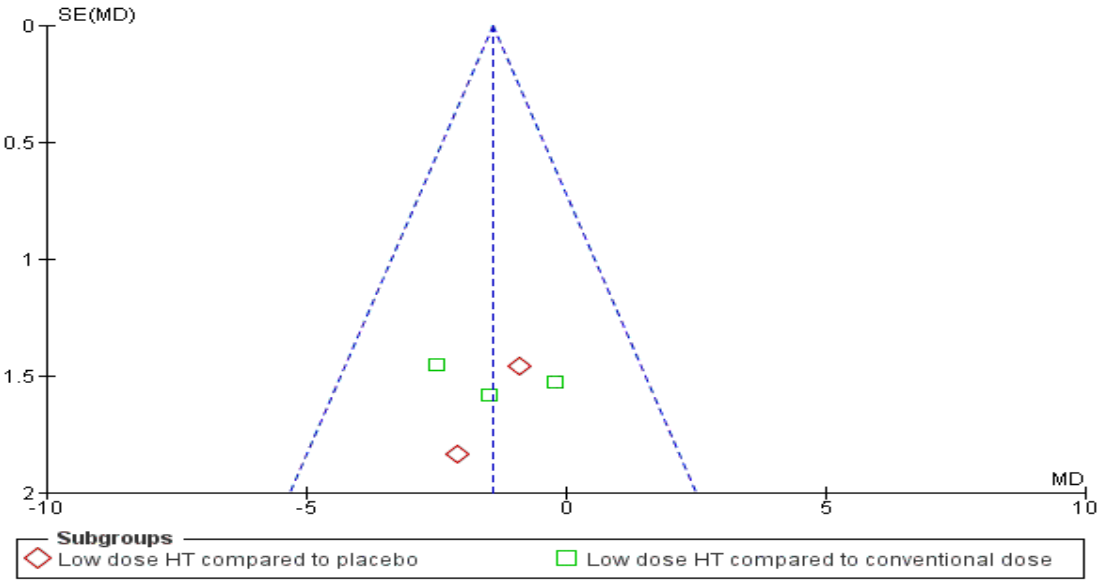
Table 2. Risk of bias summary of included studies.



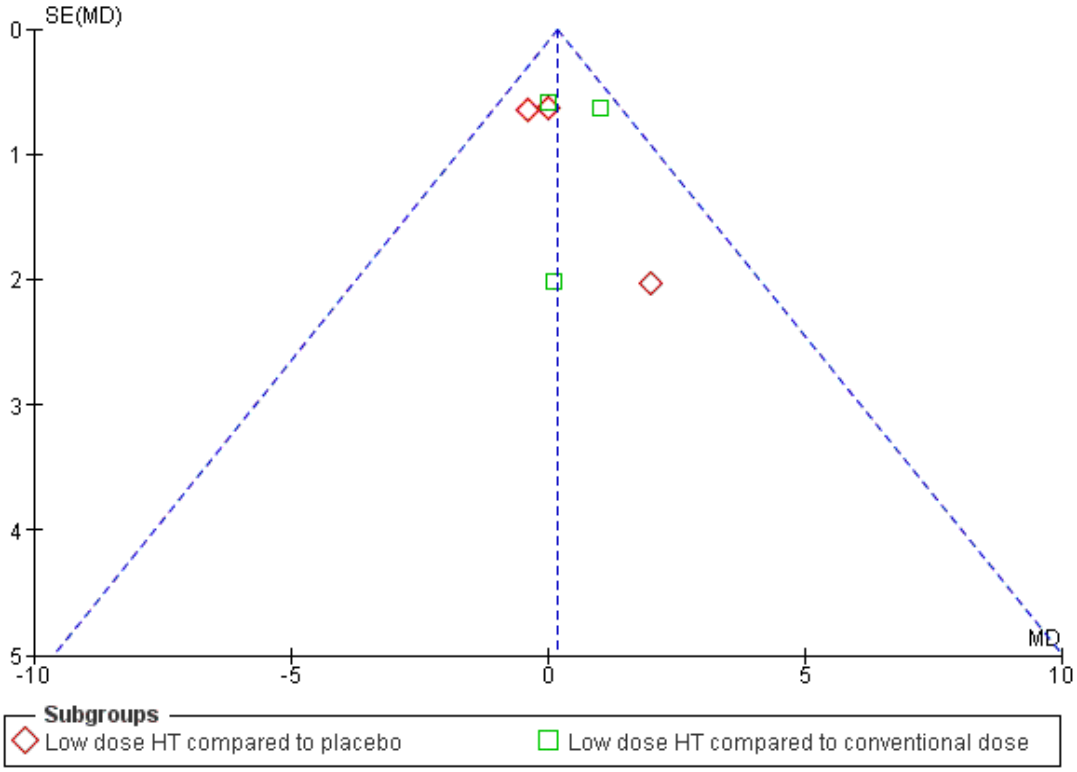
Appendix

Funnel plots for visual inspecion of risk of publication bias.

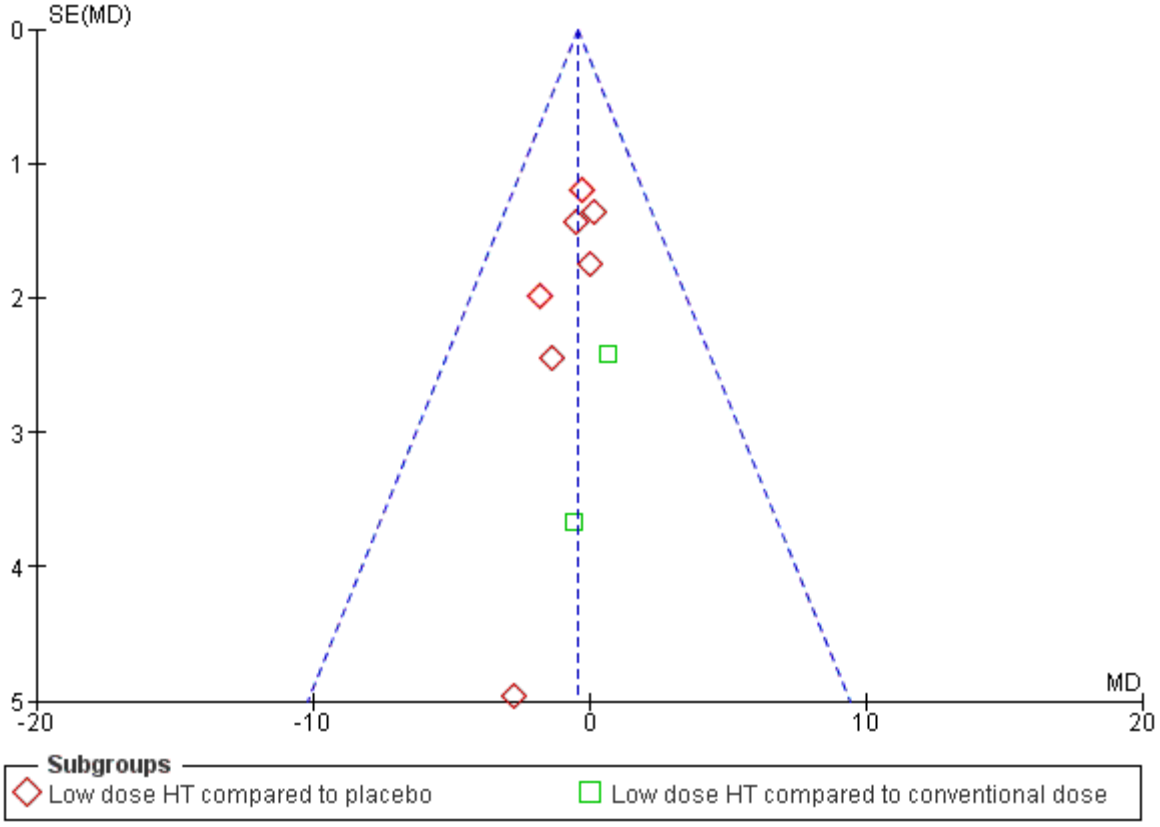
1. Weight



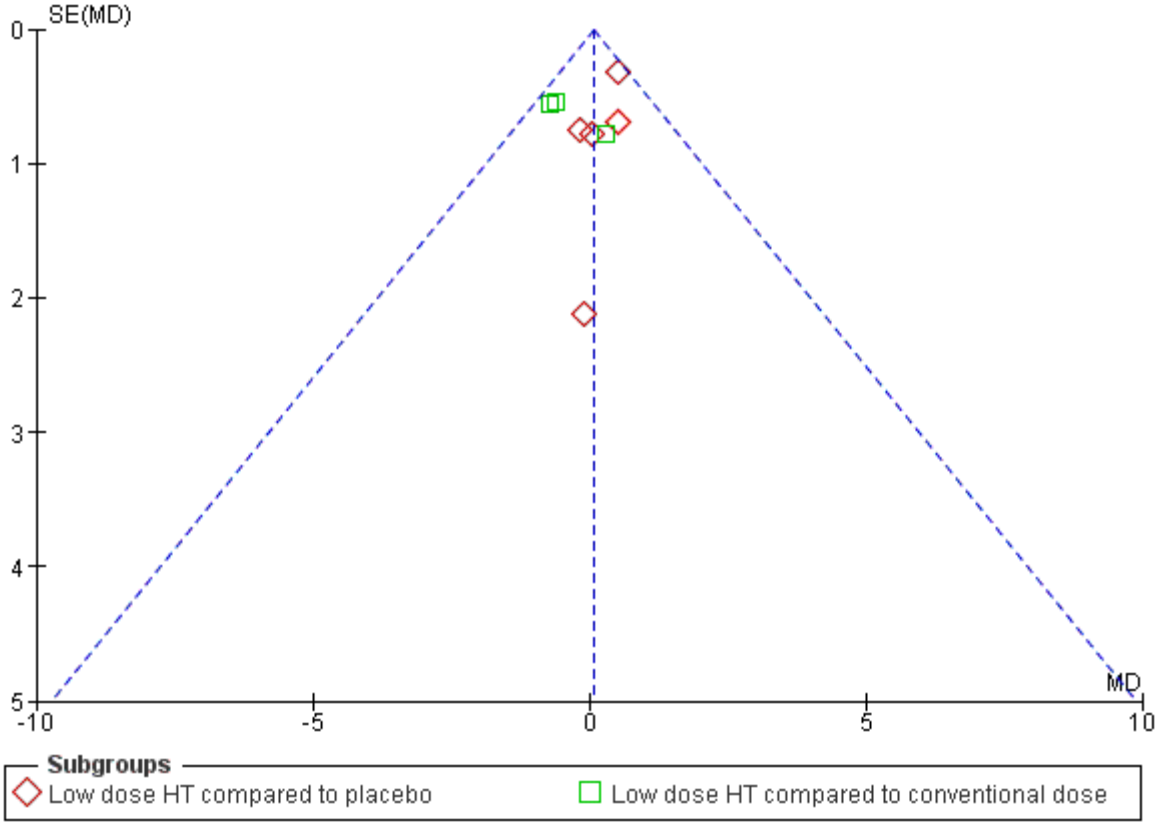
2. Body Mass Índex



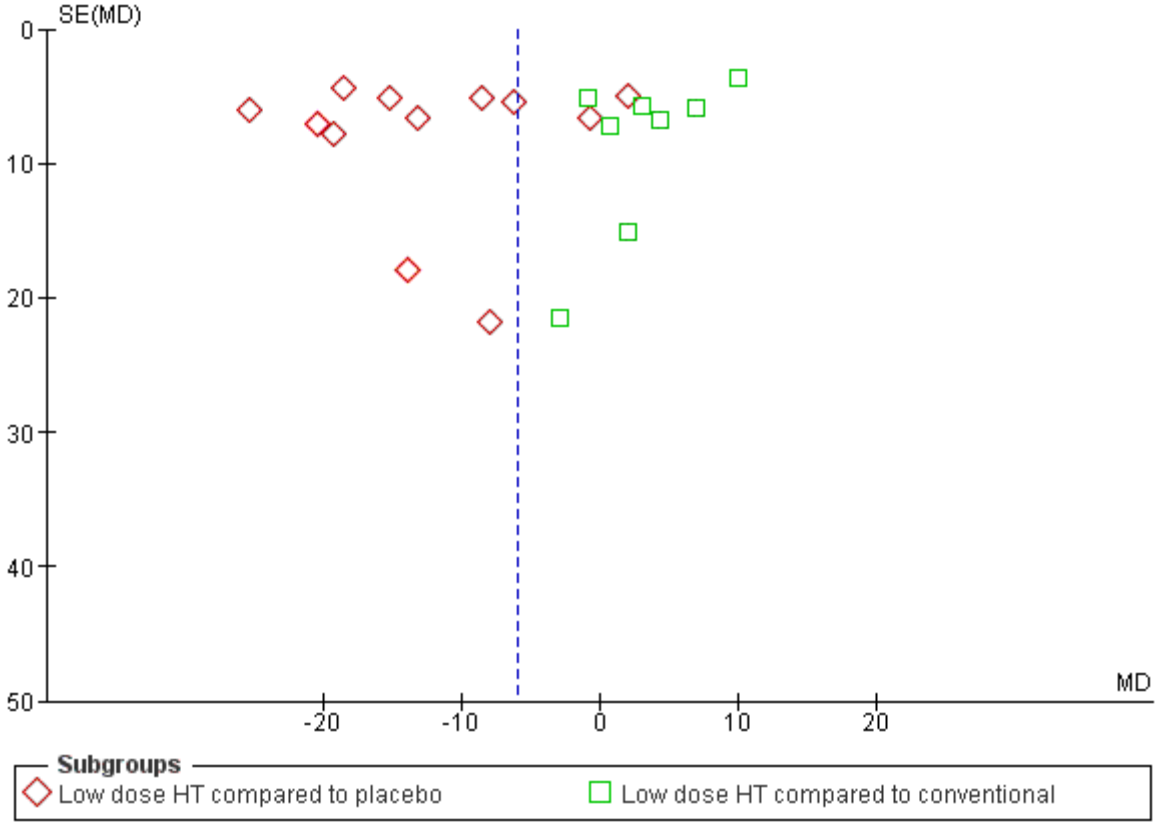
3. Mean Blood Pressure



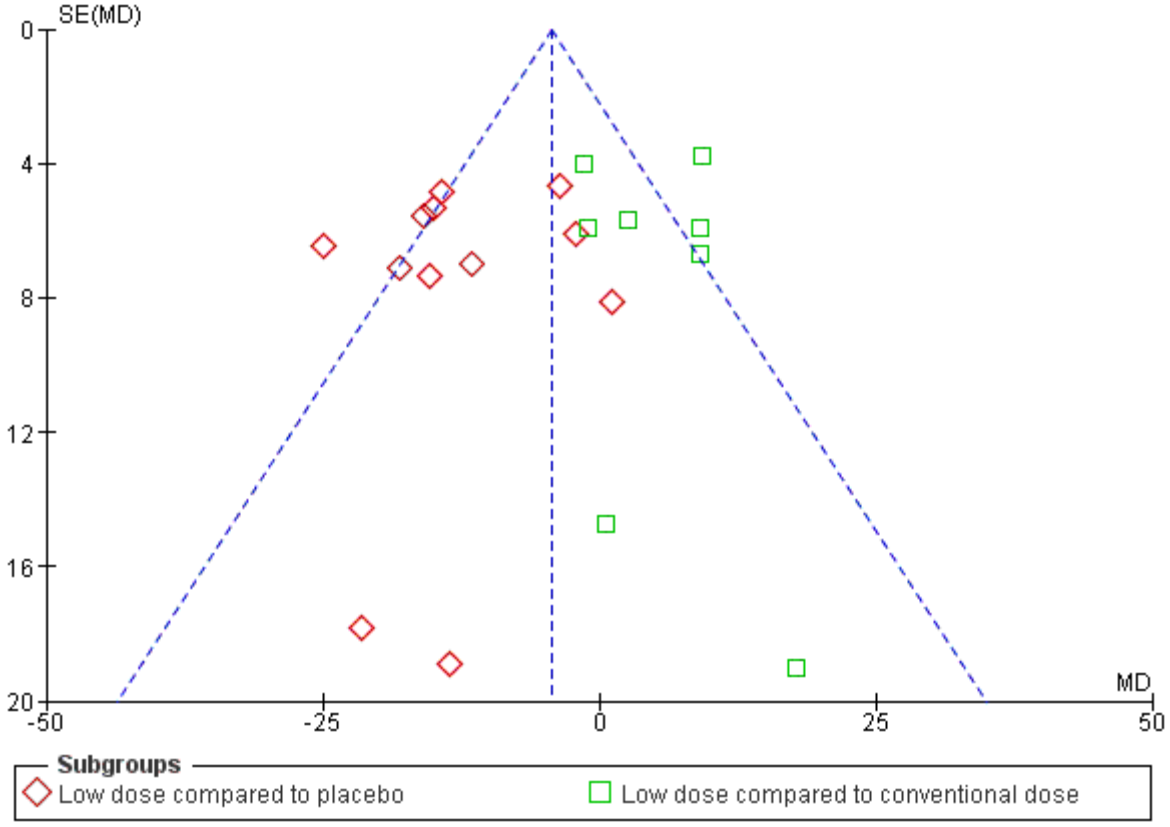
4. C- reactive protein



5. Total Cholesterol



6. LDL-c


SE(MD)
0
4
8
12
16
20
-50
-25
0
25
50
MD
Subgroups
Low dose compared to placebo
Low dose compared to conventional dose

7. HDL-c

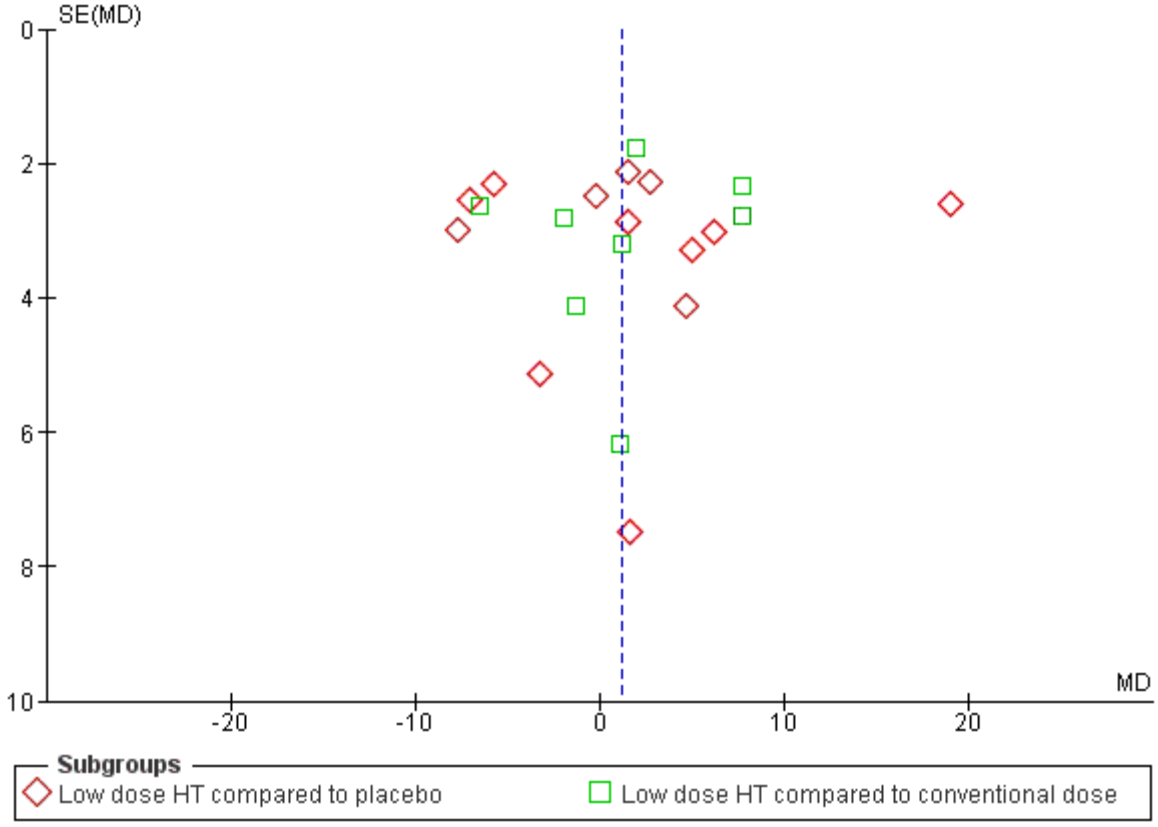

SE(MD)
0
2
4
6
8
10
-20
-10
0
10
20
MD
Subgroups
Low dose HT compared to placebo
Low dose HT compared to conventional dose

Figure 1. Flowchart. Diagram of the literature search and trials selection process.