

Universidade Federal de Pelotas
Faculdade de Medicina
Programa de Pós-Graduação em Epidemiologia

**Atividade física e composição corporal em
adolescentes da Coorte de Nascimentos de
1993 em Pelotas**

Tese de doutorado

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“Atividade física e composição corporal em adolescentes da Coorte de Nascimentos de 1993 em Pelotas”

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À minha filha Isadora que nascerá em breve

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APRESENTAÇÃO

Conforme o regimento do Programa de Pós-Graduação em Epidemiologia da Universidade Federal de Pelotas, esta tese é composta por: a) projeto de pesquisa; b) relatório do trabalho de campo; c) alterações no projeto d) relatório para imprensa; e) três artigos; f) anexos.

O projeto de pesquisa foi defendido em Abril de 2006, sendo a banca composta pelas professoras Cora Luiza Pavin Araújo e Maria Cristina Gonzalez Barbosa e Silva.

A versão apresentada nesta tese inclui as modificações sugeridas pela banca examinadora. Algumas modificações referentes aos artigos propostos inicialmente foram necessárias. O item “alterações no projeto” apresenta as razões das modificações.

PROJETO DE PESQUISA

Universidade Federal de Pelotas
Programa de Pós-Graduação em Epidemiologia
Doutorado em Epidemiologia

**DIETA, ATIVIDADE FÍSICA, GASTO ENERGÉTICO E COMPOSIÇÃO
CORPORAL EM ADOLESCENTES: UM ESTUDO DE COORTE PROSPECTIVA**

PROJETO DE PESQUISA

Doutorando: FELIPE FOSSATI REICHERT

Orientadora: ANA MARIA BAPTISTA MENEZES

Pelotas, Abril de 2006

ARTIGOS PLANEJADOS

A proposta para os três artigos exigidos pelo programa é:

- 1) Comparação do nível de atividade física determinado por questionário, sensores de movimento e água duplamente marcada;
- 2) Mudanças nos padrões de dieta, atividade física e composição corporal dos 11 aos 13 anos;
- 3) Obesidade central na adolescência e atividade física: uma revisão sistemática.

1. INTRODUÇÃO

As doenças crônicas representam atualmente a principal causa de morte em vários países. A Organização Mundial da Saúde (OMS) estima que cerca de 60% do total de mortes no mundo são atribuídas às doenças não-comunicáveis (World Health Organization¹, 2004). O Brasil está em um processo intermediário na transição epidemiológica, com crescimento proporcional das doenças crônicas em associação com manutenção e até recrudescimento de algumas morbidades infecciosas (Monteiro, 1995). Apesar dos efeitos clínicos das doenças crônicas manifestarem-se predominantemente na idade adulta, atualmente sabe-se que essas doenças podem ser pelo menos parcialmente originadas na infância e/ou adolescência (Barker, 2004; Dubos et al., 2005; Hardy et al., 2003; Singhal et al., 2003).

Entre os vários fatores de risco para doenças crônicas, destacam-se o fumo, o hábito alimentar inadequado, o baixo nível de atividade física, o excesso de peso e a obesidade (World Health Organization², 2004). Na população Finlandesa adulta, a inatividade física apresentou um risco atribuível para doenças cardiovasculares variando de 22 a 39% entre os estudos examinados (Vuori, 2001). Valores inferiores foram encontrados para tabagismo (9,5 a 32,9%), pressão sistólica $\geq 160\text{mmHg}$ (5,7 a 15,3%) e obesidade (3,7 a 7,1%) (Vuori, 2001). Estudos demonstram que a incidência desses agravos à saúde durante a infância é relativamente alta, e mais importante ainda, que existe uma tendência em manter-se o estilo de vida adotado na infância/adolescência durante a fase adulta (Dovey et al., 1998; Mikkila et al., 2005; Tammelin et al., 2003; Telama et al., 1997). Por exemplo, cerca de 80% dos adolescentes obesos também terão excesso de peso na idade adulta (Schonfeld-Warden & Warden, 1997). Além disso, a obesidade na adolescência é considerada um fator de risco para morbimortalidade cardiovascular,

independentemente do peso na idade adulta (Mossberg, 1989). Sendo assim, a obesidade na adolescência é relevante para a saúde pública tanto pelo maior risco de obesidade na idade adulta, quanto pela relação direta com desfechos desfavoráveis na própria adolescência.

Apesar da literatura atual ser consistente em demonstrar os efeitos maléficos de algumas exposições sobre o risco de doenças crônicas, a incidência destes fatores está aumentando em algumas populações. Por exemplo, estudos recentes apontam uma diminuição nos níveis populacionais de atividade física, concomitantemente a um aumento drástico na freqüência de sobrepeso na adolescência (da Veiga et al., 2004; Kimm et al., 2002). As evidências também demonstram uma crescente prevalência de dietas inadequadas e aumento do tempo despendido em atividades sedentárias de lazer (Crespo et al., 2001).

Baixos níveis de atividade física e padrões alimentares inadequados já estão entre as principais causas de diabetes tipo II, doenças cardiovasculares e alguns tipos de câncer (World Health Organization², 2004). Estudos transversais têm avaliado a associação entre estes dois fatores de risco e o índice de massa corporal (IMC); embora o IMC seja um fraco indicador de gordura entre adolescentes (Wells, 2000) e associações espúrias podem ser encontradas. Isso porque a prática regular de atividades físicas tende a promover diminuição da massa gorda e aumento da massa magra, sendo muitas vezes, incapaz de modificar a relação peso-altura, apesar dos benefícios atingidos com a diminuição de massa gorda (Laaksonen et al., 2002). Além disso, o estudo transversal não permite estabelecimento de temporalidade na associação entre atividade física, dieta e obesidade. Poucos estudos longitudinais têm investigado a relação entre dieta e atividade física com composição corporal propriamente dita.

São raras as oportunidades de se estudar a composição corporal de adolescentes longitudinalmente, particularmente em países em desenvolvimento. O presente projeto de pesquisa investigará uma sub-amostra dos adolescentes da Coorte de Nascimento de 1993 de Pelotas, RS (Barros et al., 2001; Victora et al., 2006; Victora et al., 1996). A coorte original é composta por 5249 crianças nascidas vivas, sendo que uma sub-amostra dessas ($N \sim 560$) foi acompanhada com um, três, seis meses, um, quatro e onze anos de idade. Diversos fatores de risco para obesidade foram investigados nestes acompanhamentos, entre eles dieta e nível de atividade física.

2. JUSTIFICATIVA

Apesar dos efeitos adversos da obesidade à saúde, a prevalência deste agravo está aumentando na maioria dos países e em todas as faixas etárias (Deckelbaum & Williams, 2001; Popkin & Gordon-Larsen, 2004). No Brasil, são poucos os estudos de base populacional indicando a prevalência de obesidade entre os adolescentes (Andrade et al., 2003; Magalhaes et al., 2003; Monteiro et al., 2004; Velasquez-Melendez et al., 2004). Mais escassos ainda são os estudos longitudinais sobre o tema (Monteiro et al., 2003; Victora et al., 2003). Recentemente, a associação entre taxa de crescimento na infância e composição corporal de meninos pertencentes à coorte de 1993 foi explorada (Wells¹ et al., 2005), mas estudos brasileiros incluindo adolescentes de ambos os sexos são raros.

Um dos fatores de risco não só para obesidade, mas também para doenças cardiovasculares, é a inatividade física. Entretanto, determinar o nível de atividade física em nível populacional é um desafio para os pesquisadores.

Os questionários são os instrumentos de mensuração mais utilizados em estudos epidemiológicos, apesar de suas importantes limitações, particularmente para estudos em crianças e adolescentes (Sirard & Pate, 2001). Fez-se uma busca no Medline utilizando os termos “Brazil”, “questionnaire” e “physical activity” e nenhum questionário validado para adolescentes brasileiros foi encontrado. Outros métodos em crescente utilização são os sensores de movimento (acelerômetros e pedômetros) que determinam o nível de atividade física, mas ainda é incerto se diferentes tipos e modelos desses aparelhos medem o nível de atividade física igualmente.

A OMS recomenda a prática de atividades físicas e dieta saudável tanto para tratamento como para prevenção da obesidade (World Health Organization², 2004). Poucos estudos prospectivos testaram os efeitos destas duas exposições sobre a composição corporal durante a adolescência. Dificuldades na avaliação destas variáveis, assim como da composição corporal, provavelmente desencorajam novos estudos. Algumas investigações avaliaram esta relação utilizando o delineamento transversal, impossibilitando o estabelecimento de temporalidade nas associações encontradas.

Neste contexto, a execução deste projeto de pesquisa atenderá três carências da literatura científica: 1) explorar prospectivamente a relação entre dieta, atividade física e composição corporal em adolescentes brasileiros; 2) testar a validade de um questionário de atividade física para adolescentes e; 3) comparar o nível de atividade física determinado a partir de diversos instrumentos, incluindo o uso concomitante de dois sensores de movimento (actiheart e acelerômetro).

3. REVISÃO DA LITERATURA

3.1 MENSURAÇÃO DE ATIVIDADE FÍSICA

O sedentarismo é reconhecido como um fator de risco para várias doenças crônicas, sendo assim, a aferição precisa dos níveis de atividade física é importante para: (a) determinar o nível de atividade física de populações, (b) avaliar a efetividade de intervenções e (c) estimar o efeito da atividade física sobre a incidência de doenças e/ou agravos à saúde.

Baseado no conceito de atividade física, ou seja, qualquer movimento corporal produzido pelos músculos esqueléticos que resulta em gasto energético (Caspersen et al., 1985), o padrão-ouro de avaliação pode ser considerado a observação direta dos movimentos do indivíduo. Entretanto, este método requer a avaliação constante do indivíduo por um longo período de tempo, tornando-se desvantajoso em comparação a outros métodos.

A calorimetria indireta e o método de água duplamente marcada também são considerados padrões-ouro, já que determinam, com alta precisão, o gasto energético, que é uma consequência fisiológica da atividade física. Porém, o emprego da calorimetria indireta em estudos epidemiológicos é incomum, por necessitar de um analisador de gases não portátil. A utilização de água duplamente marcada em estudos de campo vem aumentando nos últimos anos. Este método consiste na administração oral dos isótopos estáveis ^2H - ^{18}O diluídos em água. Nos 5 a 14 dias seguintes à administração, o deutério é eliminado em forma de água e o ^{18}O é eliminado tanto como água quanto como dióxido de carbono. A diferença na taxa de eliminação dos dois isótopos é uma medida da taxa de produção de CO_2 , a qual, por sua vez, é usada para

calcular o dispêndio energético. A água duplamente marcada tem a vantagem da possibilidade de administração sob condições usuais da vida dos indivíduos. Além disso, dentre as técnicas de campo disponíveis, é a mais acurada, diferindo da calorimetria em cerca de 3% (Sirard & Pate, 2001), em adultos. A maior desvantagem do método é o custo e a dificuldade na obtenção dos isótopos. Os sujeitos também devem ser re-visitados por pelo menos 3 dias para coleta de saliva ou urina. Se por um lado a obtenção do gasto energético total é acurada, não é possível obter um padrão do gasto energético por hora, dia ou mesmo a intensidade das atividades realizadas durante o período, caracterizando uma limitação do método. A obtenção de parâmetros como intensidade e duração da atividade podem ser importantes para avaliar o efeito da atividade física sobre alguns desfechos de saúde (Sirard & Pate, 2001).

Outros métodos objetivos de mensuração incluem os freqüencímetros e os sensores de movimentos (pedômetros e acelerômetros). Os freqüencímetros registram os batimentos cardíacos em uma fração de tempo. Existe uma relação linear entre o consumo máximo de oxigênio e a freqüência cardíaca, portanto, quanto maior a freqüência cardíaca de um indivíduo, mais intensa é a atividade que está sendo realizada. A maioria dos modelos possui um sensor fixado por uma cinta elástica na altura do osso externo e um receptor que é fixado no pulso, como um relógio. Neste receptor, fica armazenada a freqüência cardíaca do indivíduo e, posteriormente, estes dados são transmitidos para um computador. Os freqüencímetros são relativamente baratos, confortáveis e podem registrar os padrões de atividade física a cada minuto. A maior desvantagem do método é que em atividades leves e moderadas, a freqüência cardíaca é fortemente influenciada por outros fatores além da atividade física. Consumo de cafeína, estresse físico ou psicológico e consumo de alguns medicamentos são alguns exemplos destes fatores. Além disso, a definição da intensidade da atividade, baseada na

frequência cardíaca pode resultar em erros de classificação, pois os valores de frequência cardíaca variam muito entre os indivíduos, mesmo dentro de uma intensidade de atividade (por exemplo, moderada) (Meyer et al., 1999).

Nos últimos anos a utilização de pedômetros e acelerômetros em estudos epidemiológicos vem crescendo muito. Os pedômetros são aparelhos utilizados na cintura que medem o número de passos do indivíduo. São pequenos e requerem pouca cooperação do sujeito. Sugere-se a utilização do aparelho por dois dias consecutivos da semana e um dia do final de semana. Por avaliarem apenas os passos, não discriminam a intensidade da atividade. Além disso, atividades com grande envolvimento dos membros superiores estarão subestimadas. Não existe uma definição operacional amplamente aceita de “indivíduos ativos” ou “inativos” baseada no número de passos por dia, mas alguns trabalhos demonstraram que indivíduos que caminham mais de 10000 passos por dia apresentam proteção contra algumas doenças (Tudor-Locke & Bassett, 2004).

Os acelerômetros são similares aos pedômetros no que diz respeito ao seu tamanho e utilização. Alguns modelos de acelerômetros ainda podem ser utilizados na perna ou pulso, mas a maioria dos estudos mostra sua utilização no quadril, pois a captação de movimentos do tronco é maior. Apesar das limitações quanto à calibragem dos acelerômetros (Freedson et al., 2005), estes são superiores aos pedômetros por discriminarem a intensidade da atividade e registrarem movimentos em três planos (tronco e membros superiores e inferiores).

Um novo aparelho, chamado Actiheart (Cambridge Neurotechnology, Cambridge, UK) registra dados de frequência cardíaca e aceleração, concomitantemente (Brage et al., 2005). O Actiheart tem se mostrado superior aos acelerômetros e pedômetros quando utilizados individualmente, tanto em adultos como em adolescentes (Brage et al., 2005).

Em um estudo com 39 adolescentes de 13 anos (DP 0,3 anos), Corder et al (Corder et al., 2005) compararam o gasto energético com atividade física determinado por: a) calorimetria indireta (padrão-ouro); b) acelerômetro; c) freqüencímetro e d) Actiheart. O Actiheart apresentou maior R^2 (0,86) comparado aos demais métodos individuais ($R^2 = 0,69$ e 0,82 para acelerômetros e freqüencímetros, respectivamente). O presente estudo será um dos primeiros a utilizar os actihearts em uma amostra com número superior a 100 indivíduos (N em que o aparelho será testado =~560). Além disso, será possível comparar os dados do actiheart com acelerômetros, questionário e, em uma sub-subs-amostra, com o gasto energético determinado por água duplamente marcada.

Os métodos subjetivos de mensuração de atividade física (diários e, principalmente questionários) são os métodos mais utilizados em pesquisas com um grande número de sujeitos. Os diários consistem de uma lista com diversas atividades (variando de atividades sedentárias até vigorosas), e cada uma delas contém um número correspondente à sua intensidade. A cada 15 minutos o indivíduo deve anotar o número correspondente à intensidade da atividade que está realizando, evitando-se assim erros de recordatório. Uma grande desvantagem do diário é que a maioria deles requer um preenchimento de 3 ou 7 dias, exigindo uma enorme cooperação dos indivíduos.

Os questionários possuem algumas desvantagens em relação aos outros métodos, mas por serem logicamente fáceis e rápidos de serem aplicados, são os mais utilizados. A maior crítica aos questionários é que todos dependem, em maior ou menor grau, da lembrança do entrevistado acerca das atividades físicas realizadas no passado. Esse “passado” normalmente refere-se a uma semana habitual da vida, últimos 7 dias ou último ano. Entre crianças e adolescentes, o risco de erro de recordatório é ainda maior, particularmente pela natureza dinâmica das atividades físicas realizadas por estas

populações. Com isso, o emprego de métodos diretos de mensuração, como os acelerômetros, possui vantagem sobre os questionários.

Apesar de alguns questionários terem sido submetidos a estudos de validação, os critérios para determinar um questionário como “válido” e, particularmente, a forma de análise dos dados carecem de maior rigor científico. Devido à carência de questionários válidos para adolescentes brasileiros, um dos objetivos do presente projeto é avaliar a validade do questionário apresentado no Anexo I, tendo como padrão-ouro a água duplamente marcada e sensores de movimento.

Existem inúmeros questionários na literatura, com diferentes formas de operacionalizar “indivíduo ativo”, o que dificulta a comparabilidade do nível de atividade física entre diferentes populações. Tradicionalmente, os questionários indagavam apenas sobre atividades voluntárias, realizadas no período do lazer dos indivíduos. Apesar de existirem controvérsias acerca do efeito das atividades físicas realizadas nos outros domínios (deslocamento, trabalho e atividades domésticas) sobre a saúde (Holme et al., 1981; Hu et al., 2003; Koenig et al., 1997), a tendência atual é avaliar a atividade física total. Em 2003, foi publicado o Questionário Internacional de Atividade Física (IPAQ), em duas versões: uma longa e outra curta (Craig et al., 2003). Esse questionário é recomendado para indivíduos entre 15 e 65 anos e avalia não apenas o período de lazer, mas também as demais esferas da atividade física (deslocamento, trabalho e atividades domésticas). Apesar de sua validade ser questionável (Hallal & Victora, 2004), a OMS recomenda o seu uso, tentando assim padronizar a obtenção de dados acerca dos padrões de atividade física das populações.

Em resumo, dezenas de métodos de mensuração de atividade física estão descritos na literatura. A Figura 1 apresenta os métodos mais comuns e o grau de precisão dos instrumentos.

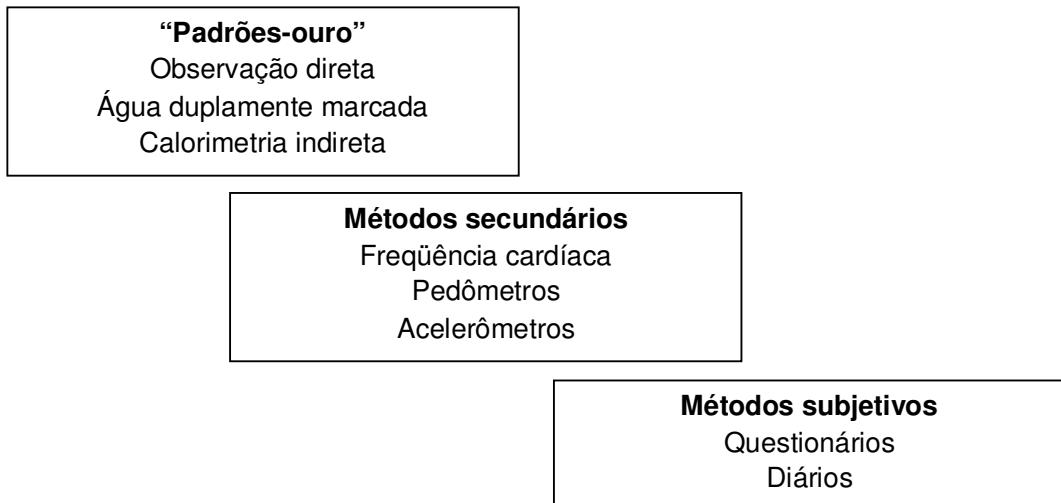


Figura 1 – Métodos de mensuração de atividade física mais utilizados em pesquisas epidemiológicas. Adaptado de: Sirard JR, Pate RR. Physical activity assessment in children and adolescents. Sports Med 2001;31(6):439-54.

3.2 NÍVEIS DE INATIVIDADE FÍSICA NA ADOLESCÊNCIA

A comparação entre os níveis de inatividade física deve sempre ser feita levando-se em conta que há diferenças na metodologia dos estudos. A quantidade mínima de atividade física que um adulto deve realizar para obter benefícios à saúde está bem descrita na literatura (Pate et al., 1995). Para adolescentes, recomenda-se a realização de 60 minutos diários de atividades moderadas ou vigorosas por pelo menos 5 dias na semana (U.S. Department of Health and Human Services & U.S. Department of Agriculture, January 2005). Porém, a base científica de onde se originou esta recomendação tem sido criticada, pois inúmeros outros benefícios podem ser alcançados com um nível de atividade física inferior a este (Twisk, 2001). Por outro lado, muitos estudos não seguem as recomendações, principalmente por dificuldades na operacionalização das mesmas.

No Brasil, apenas um estudo de base populacional com adolescentes foi localizado. Neste estudo, 960 adolescentes entre 15 e 18 anos, residentes em Pelotas foram entrevistados (Oehlschlaeger et al., 2004). O critério para inatividade física neste estudo foi realizar atividade física por menos de 20 minutos por dia e uma freqüência inferior a 3 dias por semana. A prevalência de inatividade foi de 55% entre as meninas e 22% entre os meninos. Vale ressaltar que estes valores estão subestimados, pois o ponto de corte da recomendação é mais rigoroso (300 minutos por semana de atividade física).

Valores superiores de inatividade foram encontrados em adolescentes de 15 a 18 freqüentadores de uma escola da cidade de Londrina – PR (Guedes et al., 2001). Cerca de 65% das meninas e 46% dos meninos e não alcançaram um gasto energético igual ou superior a 37Kcal/Kg/dia (ponto de corte adotado no estudo). A utilização de critérios baseados em Kcal/Kg/dia prejudica a comparação da prevalência de inatividade física com critérios baseados em minutos/semana de atividades praticadas. Valores superiores de inatividade física foram encontradas em adolescentes de 14 e 15 anos freqüentadores da rede pública de ensino de Niterói, RJ: 94% das meninas e 85% dos meninos (da Silva & Malina, 2000). Neste estudo, inatividade física foi definida como obtendo um escore inferior a três no questionário de atividade física para crianças (PAQ-C).

Estudos em outros países também indicam uma alta prevalência de inatividade física. Recentemente, nos Estados Unidos, uma amostra representativa de 3110 adolescentes de 12 a 19 anos foi avaliada quanto à baixa aptidão física (uma consequência da inatividade física e que está relacionada com aumento na incidência de doenças cardíovasculares) (Carnethon et al., 2005). Baixa aptidão física foi definida com base em valores de referência do consumo máximo de oxigênio. Cerca de 34% dos adolescentes apresentaram baixa aptidão física, sendo que não houve diferença entre os sexos. No Canadá, 2697 adolescentes de 8 escolas das zonas urbana e rural foram

classificados quanto ao nível de atividade física. Quarenta e três por cento dos adolescentes não alcançaram o critério de pelo menos 30 minutos de atividades físicas por dia.

Dados acerca do nível de atividade física de adolescentes europeus são escassos. Um estudo realizado em 15 países da União Européia entrevistou 15239 indivíduos (cerca de 1000 por país) com idade maior ou igual a 15 anos (Martinez-Gonzalez et al., 2001). Aqueles que passavam menos de 10% do tempo de lazer em atividades físicas com intensidade ≥ 4 METS* foram considerados inativos. A prevalência geral de inatividade física foi de 62%, sem diferença entre homens e mulheres (apesar de haverem diferenças em alguns países, quando analisados separadamente). Por outro lado, uma grande diferença na prevalência foi encontrada entre os países, com menores valores entre os países do norte (exemplo, Suécia 43%), enquanto Portugal apresentou a maior prevalência: 88%.

Tão ou mais importante que definir a prevalência de inatividade física é avaliar o padrão de comportamento dos indivíduos ao longo do tempo. Neste sentido, diversos estudos longitudinais são consistentes em demonstrar um declínio nos níveis de atividade física conforme o avanço da idade (Tammelin et al., 2003; Telama & Yang, 2000). Em uma coorte da Nova Zelândia, o nível de atividade física de 775 adolescentes foi avaliado dos 15 aos 18 anos (Dovey et al., 1998). Durante este período houve uma redução de 37% no tempo gasto com atividades físicas, sendo esta de magnitude semelhante entre os sexos (valor P para interação = 0,257). Aos 15 anos, as meninas realizavam, em média, 7,5 horas/semana de atividades físicas, comparado com apenas 4,3 horas/semana aos 18 anos ($p < 0,001$). Entre os meninos, os resultados foram no mesmo sentido: redução de 11,7 horas/semana aos 15 anos para 7,8 horas/semana aos 18 anos ($p <$

* MET = unidade metabólica de esforço. Um MET equivale ao consumo de O₂ em repouso e vale 3,5 ml.kg⁻¹.min⁻¹.

0,001). As causas para este declínio merecem investigações adicionais, mas alguns fatores já foram identificados. Os adolescentes que participam em vários tipos de atividade física, assim como em esportes organizados apresentam maior estabilidade nos níveis de atividade física (Aarnio et al., 2002).

3.3 MENSURAÇÃO DA COMPOSIÇÃO CORPORAL

Três fatores são apresentados na literatura como justificativas para avaliação da composição corporal na infância e adolescência: (a) o aumento na prevalência de obesidade nos últimos anos em vários países, incluindo o Brasil (Ebbeling et al., 2002); (b) o fato de que a quantidade de massa magra, em um ambiente clínico, deve ser precisamente determinada, pois serve como um índice da necessidade de calorias e líquidos que o paciente deve ingerir, em caso de alimentação artificial, e (c) a importância desta medida para a avaliação e tratamento de problemas de crescimento durante a infância e adolescência (Wells et al., 1999).

A composição do corpo humano pode ser dividida em termos de um modelo atômico (oxigênio, carbono, hidrogênio, etc.), molecular (água, proteínas, lipídeos, minerais e glicogênio), celular (sólidos e líquidos extracelulares e massa celular) ou tecidual (músculos esqueléticos, tecido adiposo, esqueleto, etc.) (Heymsfield et al., 2005). Este modelo de quatro compartimentos está apresentado na Figura 2.

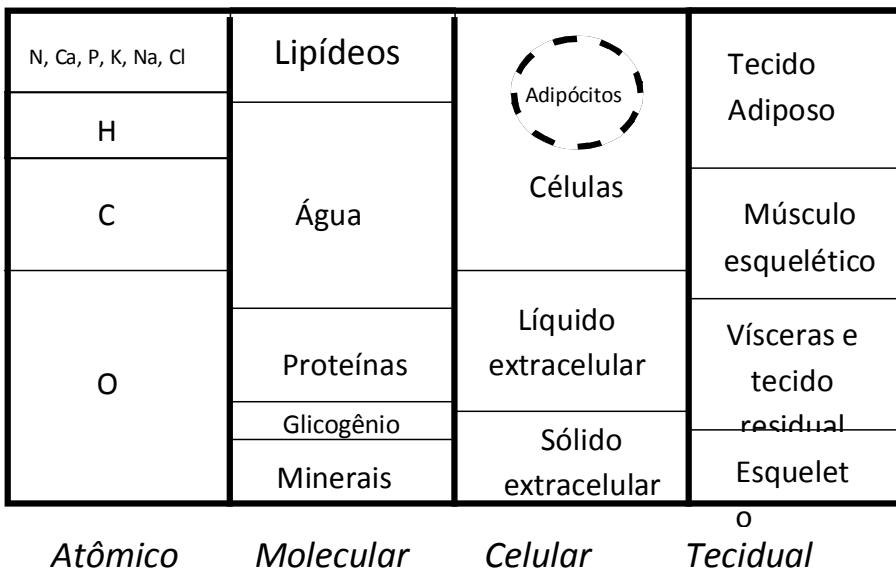


Figura 2 – Modelo de níveis de composição corporal.

Os únicos métodos de avaliação direta da composição corporal são a análise por ativação de nêutrons (disponibilidade muito limitada) e a análise química de cadáver (útil apenas para estudos com animais). Conseqüentemente, todos os métodos de determinação das massas gorda e magra utilizados em pesquisa possuem algum grau de erro de classificação. Além disso, são dependentes da idade dos indivíduos, porque as suposições dos modelos, como por exemplo, hidratação e densidade da massa magra são influenciadas pela idade e nível de maturação sexual (Goran, 1998). Algumas das técnicas mais utilizadas em pesquisas epidemiológicas para investigar composição corporal de adolescentes são brevemente discutidas a seguir.

Bioimpedância elétrica

A bioimpedância é um método rápido, barato e não-invasivo de avaliação da composição corporal. Esta técnica baseia-se no princípio de que os componentes corporais oferecem uma resistência diferenciada à passagem da corrente elétrica. A

resistência é uma função do formato do corpo, conteúdo e volume dos tecidos condutivos (Goran, 1998). Sendo assim, massa magra e massa gorda conduzem corrente de forma diferente, principalmente por diferenças na quantidade de água desses tecidos. Primeiramente, a bioimpedância estima a quantidade de água corporal total. A seguir, conhecendo-se o nível de hidratação da massa magra pode-se calcular a quantidade de massa magra e consequentemente a massa gorda. Porém, ao contrário dos adultos, o nível de hidratação da massa magra de crianças e adolescentes varia muito ($75,3 \pm 2,2\%$) (Wells et al., 1999). As equações de predição de gordura devem, portanto, levar em considerações aspectos como: sexo, faixa etária, grupo étnico e condições clínicas e consequentemente possuem uma validade externa limitada. Estudos recentes têm estimado gordura corporal regional (braços, tronco, membros inferiores) através de medidas de bioimpedância e os resultados são promissores (Fuller et al., 2002). Entretanto, mais estudos são necessários nesta área.

Por ser um método dependente do grau de hidratação do indivíduo, alguns cuidados devem ser tomados para que a estimativa tenha um baixo erro: o indivíduo não deve ingerir alimentos e/ou bebidas nem praticar atividades físicas vigorosas algumas horas antes da avaliação. Além disso, o consumo de diuréticos pode influenciar nos valores.

Absortometria de raios-X de dupla energia (DEXA)

O DEXA foi inicialmente desenvolvido para avaliar o conteúdo mineral ósseo e, hoje em dia, também é considerado um dos padrões-ouro para avaliação da composição corporal. Este método baseia-se na emissão de baixas doses de dois níveis de energia. Os raios-X são absorvidos pelo corpo e o resultado é dado pela razão entre os dois

níveis de energia. Ao final, tem-se um modelo de três componentes de composição corporal: mineral ósseo, massa magra e massa gorda (Goran, 1998).

A maior vantagem é ser possível determinar não apenas a quantidade de gordura total, mas também de algumas regiões do corpo. Também é considerado um método seguro (baixa radiação) e relativamente rápido (cerca de 20 minutos por avaliação).

Entre as desvantagens do método destaca-se o custo, a falta de praticidade (o indivíduo deve ir até um laboratório para ser avaliado), e a necessidade de um técnico treinado para operar o equipamento.

Diluição de deutério (D_2O)

A diluição de deutério é considerada o método mais acurado para investigar composição corporal em adolescentes (Wells et al., 1999). Esse método é ideal para estudos epidemiológicos, sendo uma técnica não-invasiva e necessitando um esforço mínimo do indivíduo. Assim como a bioimpedância elétrica, este método primeiramente fornece a quantidade de água total corporal.

Os passos para determinação da composição corporal através deste método são: uma amostra de saliva (ou urina) seguido da administração de 0,05g de deutério por quilograma de peso corporal (para adolescentes) diluídas em água (Wells² et al., 2005). Aguarda-se então um período de aproximadamente 5 horas, necessário para o equilíbrio do isótopo com a água corporal, e então coleta-se novamente saliva. Neste momento é possível calcular a quantidade corporal de água utilizando princípios de diluição (Wells² et al., 2005).

Existem dois métodos para cálculo da quantidade de água corporal total usando isótopos estáveis: o método de platô e o *back extrapolation*. Ambos os métodos são falhos, com

o método de platô produzindo valores maiores que o *back extrapolation*, particularmente em crianças menores do que 2 anos (Davies & Wells, 1994). Os dois métodos tendem a dar valores muito similares, conforme o avanço da idade.

Entre as desvantagens da diluição dos isótopos em água destacam-se o alto custo associado às análises dos isótopos (necessidade de um espectrômetro de massa) e o tempo para obtenção dos dados.

Antropometria

Índice de Massa Corporal (IMC)

Apesar da existência de métodos sofisticados de avaliação da obesidade, o IMC continua a ser a medida antropométrica mais utilizada. Entretanto, o IMC não avalia a composição corporal. De fato, para um único valor de IMC, pode ser encontrada uma ampla variação na quantidade de gordura corporal (Wells, 2000; Wells et al., 2002), especialmente em adolescentes em diferentes estágios de maturação sexual.

O IMC é calculado dividindo-se a massa corporal total pela estatura ao quadrado (Kg/m^2), ou seja, não há distinção entre as massas gorda e magra. A elevação da altura ao quadrado é necessária para ajustar o peso em relação à altura de forma que a correlação entre o índice e a altura seja mínima. Sendo assim, qualquer aumento no valor do índice seria devido a aumentos na massa corporal dos indivíduos. Contudo, durante a adolescência, em ambos os sexos, o aumento no IMC deve-se principalmente a aumentos na massa magra, ao invés da quantidade de gordura corporal (Maynard et al., 2001).

Para adultos (>19 anos), valores de $\text{IMC} \geq 25$ e $\geq 30\text{Kg}/\text{m}^2$ indicam sobrepeso e obesidade, respectivamente. Entre adolescentes (idades entre 10 e 19 anos), os valores

diferem conforme a idade e o sexo. Para meninos de 13 anos (idade da maioria dos adolescentes deste projeto), sugere-se como ponto de corte para sobre peso e obesidade os valores de 21,9 e 26,8Kg/m², respectivamente (Cole et al., 2000). Para as meninas da mesma idade os valores são 22,6 e 27,8Kg/m², respectivamente.

Entre as grandes vantagens deste método (IMC), destaca-se a facilidade na obtenção das medidas e a possibilidade de comparação entre estudos. Além disso, vários estudos epidemiológicos utilizam a massa corporal e altura auto-relatada pelos indivíduos, sendo este um método considerado válido em algumas populações (Bolton-Smith et al., 2000; Silveira et al., 2005).

Dobras cutâneas e perímetros

A mensuração de dobras cutâneas e perímetros é uma maneira rápida e barata de se obter estimativas da composição corporal dos indivíduos. O método envolve a utilização de modelos que relacionam a soma de dobras e diâmetro de perímetros com a quantidade de gordura corporal. Uma das suposições destes modelos é que a gordura subcutânea, mensurada pelo método, é proporcional à gordura total, o que é uma limitação importante, porque a distribuição de gordura pode variar entre os indivíduos e no mesmo indivíduo (Goran, 1998). Outra limitação importante do método é a baixa reprodutibilidade das medidas, o que pode ser atenuado por um extensivo treinamento acerca das técnicas corretas de mensuração.

Diversas equações, para populações e subgrupos populacionais têm sido desenvolvidas e validadas contra métodos mais precisos, como DEXA, por exemplo. Entretanto, vale ressaltar que algumas destas equações são criticadas pelo alto erro nos valores encontrados (Wells et al., 1999).

Praticamente todas as equações transformam as medidas de dobras cutâneas e perímetros para um percentual de gordura corporal (total de massa gorda dividido pela massa corporal total). Porém, já está bem demonstrado que esta transformação carece de respaldo científico (Wells, 2001; Wells & Victora, 2005). Atualmente, sugere-se que a massa gorda seja ajustada para a massa magra elevada a um exponencial que depende das dobras cutâneas mensuradas (Wells, 2001; Wells & Victora, 2005).

No Brasil, alguns estudos sobre obesidade têm utilizado medidas de dobra cutâneas (Chiara et al., 2003; Monteiro et al., 2000; Victora et al., 2003). Na cidade do Rio de Janeiro, 502 adolescentes de 12 a 18 anos foram avaliados quanto ao risco de obesidade baseado em medidas de dobras cutâneas e IMC (Chiara et al., 2003). A dobra cutânea subescapular identificou mais adolescentes em risco de obesidade que o IMC.

A adiposidade central é um forte preditor de doenças cardiovasculares (Karelis et al., 2004). Na ausência de técnicas de campo mais factíveis, o uso de perímetro da cintura e quadril tem sido utilizado para estimação da gordura visceral. Em estudos de validação, o método de ressonância magnética normalmente é usado como padrão-ouro. Em adolescentes, o uso apenas do perímetro da cintura apresenta-se como uma boa estimativa da quantidade de gordura visceral, explicando cerca de 65% da variância (Brambilla et al., 2006). Para adultos, existem valores de referência para serem utilizados, mas para adolescentes estes pontos de corte inexistem. Na ausência de um critério definido, alguns estudos utilizaram arbitrariamente os percentis 91 e 98 para definir sobrepeso e obesidade, respectivamente (Garnett et al., 2005; McCarthy et al., 2003). A justificativa é que estes valores são próximos dos percentis utilizados para definir obesidade e sobrepeso com base no IMC.

Comparação entre os métodos

Alguns estudos comparando diferentes métodos estão disponíveis na literatura. Dentre as técnicas mais factíveis de serem empregadas em estudos epidemiológicos, a diluição de isótopos é a mais eficaz (Wells et al., 1999). Bioimpedância elétrica e dobras cutâneas são métodos com menor reproduzibilidade, e a acurácia deste último método é dependente do nível de treinamento do avaliador. Além disso, em alguns estudos de validação de equações de predição de gordura corporal, o método definido como “padrão-ouro” possui um alto erro, o que comprometeria o estudo. Uma das grandes limitações quanto aos estudos de validação, diz respeito às análises estatísticas. Alguns estudos utilizaram apenas o coeficiente de correlação para avaliar a concordância entre métodos (Steinberger et al., 2005), entretanto esta ferramenta estatística é reconhecidamente ineficaz para atender este objetivo (Hallal & Victora, 2004). Autores que utilizaram outras técnicas de análises, como por exemplo Bland & Altman, demonstram concordância baixa ou moderada entre os métodos, tanto em crianças e adolescentes (Brambilla et al., 2006; Piers et al., 2000; Wells et al., 1999) como em adultos (Piers et al., 2000). Além disso, o mesmo método pode apresentar resultados bem diferentes, dependendo da equação utilizada. Por exemplo, a estimativa de água corporal total através de diluição de deutério pode apresentar erros-padrão da estimativa entre 7,8 a 11,2%, dependendo da equação utilizada (Wells² et al., 2005).

Apesar das limitações das técnicas antropométricas em determinar com exatidão a quantidade de massa gorda, a utilização padronizada da mesma medida ao longo do tempo é de grande utilidade para verificar tendências temporais nos padrões de composição corporal das populações.

3.4 RELAÇÃO ENTRE DIETA, ATIVIDADE FÍSICA E COMPOSIÇÃO CORPORAL

A obesidade na adolescência é fator de risco para vários desfechos adversos à saúde na idade adulta (DiPietro et al., 1994; Eckel & Krauss, 1998), assim como na própria adolescência (McLennan, 2004). Além disso, indivíduos que se tornam obesos na adolescência, tendem a persistirem com um alto peso na idade adulta (Schonfeld-Warden & Warden, 1997).

Nas últimas décadas, a prevalência de obesidade na adolescência aumentou na maioria dos países (Berkey et al., 2000). Na população adulta brasileira estima-se um aumento de pelo menos 100% na freqüência de $\text{IMC} \geq 25/\text{kg}^2$ nos últimos 30 anos (Mendonça & dos Anjos, 2004). Entre os adolescentes, o aumento na prevalência de sobrepeso (baseado no IMC) entre os anos de 1975 e 1997 foi ainda maior: quase 300% entre as meninas (de 5,8 para 15,3%) e 400% entre os meninos (de 2,6 para 11,8%) (da Veiga et al., 2004). Na região sudeste, estima-se que cerca de 17% dos adolescentes apresentem sobrepeso.

Apesar da obesidade ter uma etiologia multidimensional, mudanças nos padrões de dieta e diminuição no tempo gasto em atividades físicas são apontados como as principais causas deste aumento abrupto (Ebbeling et al., 2002).

Alguns estudos têm verificado o efeito de ambas as exposições sobre a composição corporal. Um quadro com um resumo de artigos revisados sobre este tópico está apresentado ao final deste item da revisão bibliográfica (quadro 1). A maioria dos estudos apresentados no quadro 1 foram realizados com amostras de adultos e residentes em países desenvolvidos. Estudos com adolescentes estão descritos a seguir no texto.

O efeito de atividade física e ingestão calórica total, assim como de fibras e gordura separadamente sobre mudanças no IMC ao longo de um ano foram explorados recentemente (Berkey et al., 2000). No *baseline* as 6149 meninas e 4620 meninos acompanhados tinham entre 9 e 14 anos. Atividade física esteve negativamente associada com aumentos no IMC entre as meninas ($-0,0284 \pm 0,0142\text{Kg/m}^2/\text{hora/dia}$). Um aumento na quantidade total de calorias ingerida foi a única variável associada com aumento no IMC em ambos os sexos. Por outro lado, nem a ingestão de fibras, nem de gordura se mostrou associado com aumentos no IMC. Algumas limitações importantes do trabalho devem ser consideradas. Primeiro, a falta de associação entre IMC e atividade física entre os meninos pode ser explicada pelo fato da atividade física aumentar a massa magra. Segundo, houve um alto percentual de perdas e recusas (32% entre as meninas e 42% entre os meninos) indicando um possível viés de seleção. Por fim, os dados foram coletados a partir de questionários enviados ao domicílio dos participantes, e peso corporal e altura foram auto-referidos.

Estudo semelhante, mas com uma metodologia mais rigorosa, foi realizada com adolescentes gregos de 12 a 14 anos (Koutedakis et al., 2005). O tempo de acompanhamento foi de 2 anos, e durante este período três avaliações foram realizadas (*baseline* e após 12 e 24 meses). O número de indivíduos na primeira, segunda e terceira avaliação foi 210, 204 e 198, respectivamente. Percentual de gordura foi estimado a partir de dobras cutâneas. Dados acerca do nível de atividade física (questionário), aptidão física (teste físico) e calorias ingeridas (diário de 7 dias, com fotos dos alimentos mais consumidos em diferentes tamanhos de porções para auxílio no preenchimento do instrumento) foram coletados. Atividade física e aptidão física estiveram negativamente associadas com gordura corporal, enquanto a ingesta calórica não demonstrou nenhuma associação.

Apesar dos resultados encontrados por estes estudos, diversos outros, incluindo estudos de revisão destacam a importância de uma dieta saudável na prevenção e tratamento da obesidade (Astrup, 2001; Goran & Treuth, 2001; Holmes & Kwiterovich, 2005).

A associação entre dieta e atividade física com obesidade visceral não é tão clara, mas alguns estudos sugerem que estas exposições estão associadas ao desfecho (Rennie et al., 2003; van Lenthe et al., 1998). A obesidade visceral está associada à resistência insulínica, baixos níveis do colesterol HDL e altos níveis de triglicerídeos (Karelis et al., 2004). Este tipo de obesidade também é mais persistente: Um estudo de coorte acompanhou 342 crianças dos 7-8 até os 12-13 anos (Garnett et al., 2005). Ao final, 79% das crianças que tinham sobrepeso ou obesidade (determinados por IMC) aos 7 anos continuaram a apresentar aos 12 anos. Quanto à obesidade central (estimada por perímetro da cintura), o valor foi de 88%.

Existem poucos estudos experimentais sobre o tema, sendo que a maioria deles está apresentada em um estudo de revisão (Watts et al., 2005). Em um destes trabalhos, foi demonstrado que um programa de exercícios pode amenizar a disfunção endotelial, além de diminuir gordura total e abdominal (Watts et al., 2004). Disfunção endotelial é a primeira manifestação mensurável de aterosclerose. Em outro estudo, foi demonstrado que a prática de atividade física em níveis ligeiramente superiores que as recomendações, pode ser um efetivo tratamento para diminuição da obesidade visceral (Slentz et al., 2005).

Quadro 1 – Resumo de artigos sobre dieta, atividade física e sobrepeso/obesidade.

Número da Referência	País	Objetivo	Aspectos metodológicos	Principais resultados
80	Holanda	Verificar a associação, entre outros, de AF e consumo calórico com obesidade central	Coorte; 84 homens e 98 mulheres acompanhados dos 13 aos 27 anos	Associação negativa AF E dobra cutânea subescapular em mulheres. Consumo calórico não associou-se com obesidade central.
45	Finlândia	Associação entre AF de lazer e nível de aptidão física e desenvolvimento de SM	Coorte; 612 homens entre 42 e 60 anos no <i>baseline</i>	Indivíduos ativos e com melhor aptidão física desenvolveram menos SM. Um efeito dose-resposta em relação à intensidade da AF praticada e incidência de MS foi observado.
70	EUA	Verificar o efeito da AF sobre quantidade de gordura abdominal	ECR; 175 indivíduos entre 40 e 65 anos inicialmente sedentários, com sobrepeso ou obesidade, dislipidemia.	Quantidade de AF equivalente as recomendações atuais previne aumento de gordura subcutânea e visceral na região abdominal. Quantidade de AF ligeiramente superior à recomendação, reduz a quantidade de gordura abdominal, sem necessidade de restrição calórica.
7	EUA	Estudar a associação entre alterações no IMC entre 1997-98 com alterações em AF e atividades sedentárias no mesmo período	Coorte; 11887 adolescentes entre 10 e 15 anos; dados coletados por questionários enviados aos domicílios	Aumento no nível de AF de 1997 a 1998 resultou em decréscimo no IMC em meninas e meninos com sobrepeso. Aumento em tempo de TV/vídeo/video game resultou em aumentos no IMC apenas em meninas

65	Inglaterra	Verificar a relação entre AF vigorosa e moderada e MS	Transversal; 5153 adultos (45-68 anos)	Atividades vigorosas e moderadas estão associadas a menor probabilidade de MS, entretanto a magnitude do efeito é maior para AF vigorosas.
61	EUA	Examinar a relação entre consumo de lacticínios com peso relativo e percentual de gordura	Coorte; 178 meninas de 8-12 anos não-obesas e na pré-menarca foram acompanhadas até 4 anos após menarca	Os resultados não demonstraram associação entre consumo de lacticínios e aumento de escore Z de IMC nem aumento de percentual de gordura.
40	Canadá	Verificar a associação entre AF de lazer, dieta e sobrepeso e obesidade	Transversal; 5890 adolescentes de 11-16 anos; amostra representativa do país	Não houve associação entre dieta e sobrepeso ou obesidade. Indivíduos ativos apresentaram menos sobrepeso e obesidade que indivíduos inativos.

Abreviaturas:

AF – atividade física

SM – síndrome metabólica

ECR – ensaio clínico randomizado

4. OBJETIVOS

OBJETIVOS GERAIS

Descrever as alterações, dos 11 aos 13 anos, nos padrões de dieta, atividade física e composição corporal de adolescentes pertencentes a uma coorte prospectiva de base populacional;

Comparar os níveis de atividade física determinados por questionário, acelerômetros, actihearts e água duplamente marcada nestes adolescentes;

OBJETIVOS ESPECÍFICOS

Neste grupo de adolescentes:

Verificar a associação de medidas antropométricas (IMC e dobras cutâneas), dieta e nível de atividade física aos 11 anos com os seguintes desfechos aos 13 anos:
composição corporal;
medidas antropométricas (perímetros, dobras cutâneas e IMC);
padrão de dieta e;
nível de atividade física.

Estimar o gasto energético diário em uma sub-amostra, através do método de água duplamente marcada;

Avaliar a gordura corporal total e abdominal e fatores associados;

Comparar os níveis de gordura corporal determinados por diluição de deutério, dobras cutâneas, perímetro abdominal e IMC;

5. HIPÓTESES

Haverá uma forte tendência em se manter o estilo de vida dos 11 aos 13 anos, sendo que a prevalência de inatividade física e hábitos inadequados de alimentação deverá ser ainda maior aos 13 anos do que aos 11 anos;

Haverá uma alta concordância no nível de atividade física determinado pelos diferentes métodos (questionário, acelerômetro, etc.);

Os seguintes fatores serão risco para inatividade física aos 13 anos:

Ser inativo aos 11 anos;

Ter maior somatório de dobras cutâneas aos 11 anos;

Ter maiores níveis de gordura corporal central aos 13 anos.

Maiores índices de gordura corporal (total e abdominal) aos 13 anos serão encontrados em adolescentes:

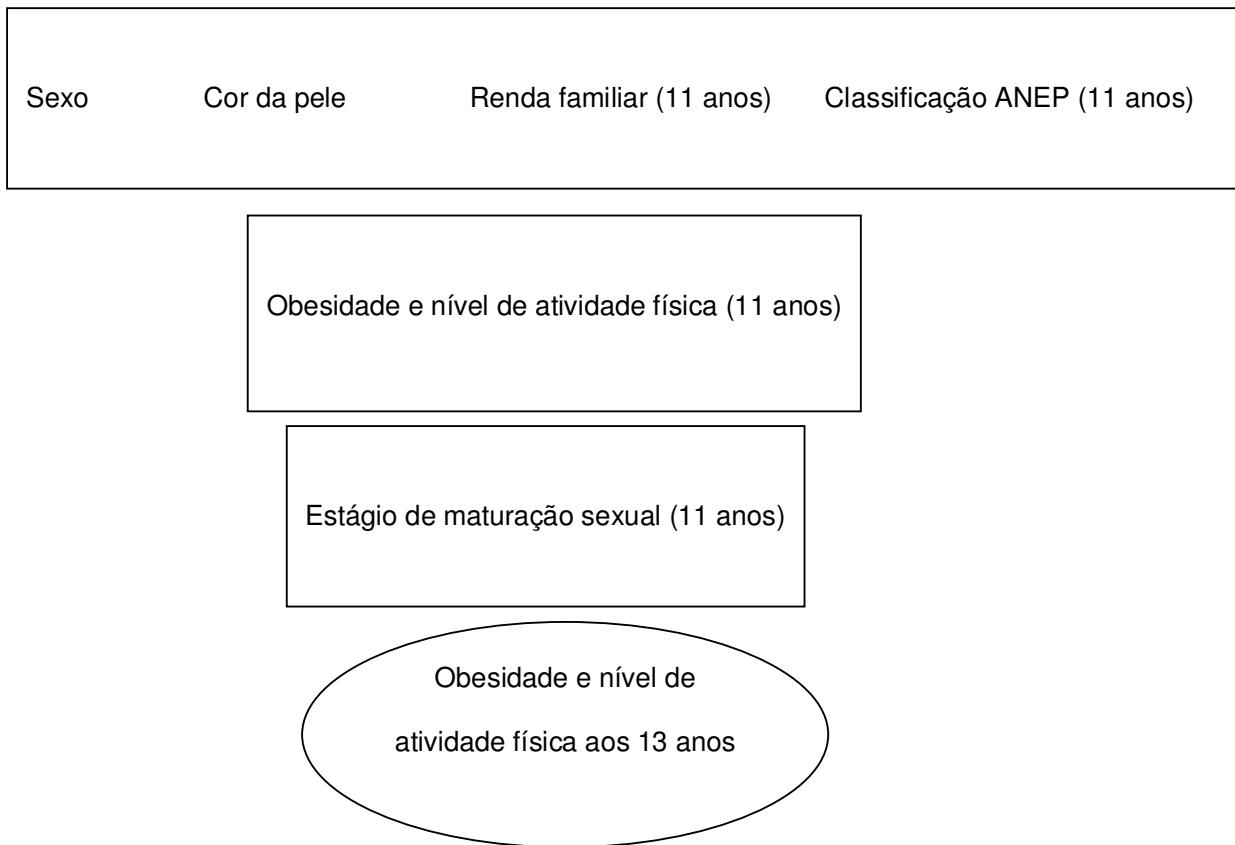
Fisicamente inativos aos 11 anos;

Com uma dieta rica em gorduras aos 11 anos;

De pior nível socioeconômico aos 11 anos.

As diferentes técnicas de determinação de gordura corporal apresentarão alta concordância para a maioria dos indivíduos;

6. MODELO CONCEITUAL



O modelo conceitual apresenta fatores sociodemográficos em um nível distal de causalidade. A literatura é consistente ao demonstrar que meninas possuem mais chance de serem sedentárias no tempo de lazer (Guedes et al., 2001; Trost et al., 2002). Além disso, indivíduos de cor da pele não-branca e pior nível socioeconômico possuem menos conhecimento sobre os benefícios da atividade física (Domingues et al., 2004), e por isso, podem ser mais sedentários e consequentemente mais obesos.

Diversos estudos apresentam uma tendência em manter determinados comportamentos ao longo da vida. Por exemplo, crianças sedentárias são mais propensas a tornarem-se

adolescentes sedentários e estes, por sua vez, de tornarem-se adultos sedentários (Tammelin et al., 2003; Telama & Yang, 2000; Telama et al., 1997). A mesma tendência é observada quanto aos hábitos alimentares inadequados, e, portanto, obesidade (DiPietro et al., 1994; Mossberg, 1989; Reilly et al., 2005). Sendo assim, os níveis de obesidade (estimados por dobras cutâneas e IMC) e atividade física aos 11 anos de idade, são prováveis indicadores destas características aos 13 anos.

Apesar dos fatores acima citados serem o foco de interesse do presente projeto, o estágio de maturação sexual é altamente determinante do grau de obesidade do adolescente. A menarca ocorre em meninas com maior acúmulo de gordura, enquanto que meninos com maturação sexual precoce apresentam menor acúmulo de gordura. Sendo assim, o estágio de maturação sexual é um importante e complexo fator que deve ser considerado nas análises de obesidade durante a adolescência (World Health Organization, 1995).

7. METODOLOGIA:

7.1. Delineamento

Estudo de coorte prospectivo. Dentre os delineamentos observacionais, a coorte prospectiva é o melhor tipo de estudo para investigar as alterações nos padrões de comportamento durante a adolescência, possibilitando estabelecer uma relação temporal entre as exposições e os desfechos. As análises de comparabilidade de instrumentos para determinar nível de atividade física utilizarão apenas os dados do presente acompanhamento, podendo ser considerado um estudo transversal.

7.2. População-alvo e amostra

Este projeto está vinculado à coorte de nascimentos de 1993 da cidade de Pelotas, RS (Victora et al., 2006). Resumidamente, todas as crianças nascidas vivas na cidade de Pelotas no ano de 1993 cujas famílias residiam na cidade na época (N=5265) foram convidadas a fazerem parte de um estudo perinatal, sendo que 5249 efetivamente participaram do estudo (16 recusas; percentual de não resposta de 0,3%).

A tabela 1 descreve os principais acompanhamentos da coorte de 1993. Sub-amostras dessas crianças foram visitadas com um, três e seis meses, um e quatro anos de idade. Aos 11 anos, todos os participantes da coorte foram procurados para um novo acompanhamento, sendo que 87,5% destes foram entrevistados. Dos 655 indivíduos amostrados em todos os acompanhamentos já realizados (um, três e seis meses, um, quatro e 11 anos), 568 foram localizados no acompanhamento de 2004. Estes 568 adolescentes são os elegíveis para este projeto. Destes, ainda será amostrado uma sub-amostra de cerca de 30 adolescentes para avaliação do gasto energético. A amostragem para seleção destes 30 adolescentes será estratificada pelo nível socioeconômico aos 11 anos. Deste modo, espera-se uma grande amplitude no nível de atividade física dos adolescentes.

7.3. Cálculo do tamanho de amostra

Mantendo o nível de confiança a 95%, a amostra a ser visitada (N~560) permitirá detectar prevalências variando de 20 a 70% com um erro máximo de quatro pontos percentuais. Com um poder de 80%, será possível detectar razões de prevalência

maiores ou iguais do que 2, com freqüência de desfechos variando de 20 a 70%, e prevalências de exposição variando de 10 a 80%.

7.4. Definição operacional das variáveis

Inatividade física – menos que 300 minutos de atividades físicas por semana (as aulas de Educação Física na escola não serão computadas) (U.S. Department of Health and Human Services & U.S. Department of Agriculture, 2005). O escore será calculado a partir do questionário sobre atividade física (anexo I).

Consumo excessivo de gordura – escore igual ou maior a 27 pontos no questionário Block (anexo I; questões 31 a 45).

Consumo inadequado de fibras – escore menor que 20 no questionário Block (anexo I; questões 46 a 57).

Sobrepeso / obesidade – será definido utilizando o IMC, tendo como referência o critério da OMS (World Health Organization, 1995).

Obesidade central – não há uma definição de obesidade central para adolescentes, entretanto, uma perímetro abdominal maior ou igual ao percentil 98 da distribuição da amostra tem sido utilizada em alguns trabalhos (Garnett et al., 2005) e a mesma definição será adotada no presente projeto.

Tabela 1. Principais acompanhamentos da coorte de nascimentos de 1993 da cidade de Pelotas.

Ano	Idade	Estratégia amostral	<i>Indivíduos elegíveis (N)</i>	<i>Taxa de resposta*</i> (%)
1993	Nascimento	Todos os nascimentos ocorridos em 1993 nas cinco maternidades de Pelotas	5.265	99,7%
1993-4	1 mês	Amostra sistemática de 13% de todos os participantes	655	99,1
1993-4	3 meses	Idem à visita anterior	655	98,3
1993-4	6 meses	Todas as crianças com baixo peso ao nascer (<2,500 g) e 20% das restantes (incluindo aqueles visitados nos acompanhamentos de um e três meses)	1.460	96,8
1994-5	12 meses	Idem à visita anterior	1.460	93,4
1997-8	4 anos	Idem à visita anterior	1.460	87,2
2004-5	11 anos	Todos os participantes da coorte	5.249	87,5

* Percentual dos indivíduos da coorte original elegíveis para os acompanhamentos e que foram entrevistados ou sabia-se que havia falecido.

7.5. Variáveis independentes

As variáveis independentes, assim como sua operacionalização e tipo estão apresentadas na Tabela 2. A variável *inatividade física*, definida como *dependente* acima, será analisada como *independente* quando o desfecho for obesidade.

Tabela 2 – Operacionalização e tipo das variáveis independentes estudadas.

Variável	Operacionalização	Tipo
Sexo	Masculino / feminino	Categórica dicotômica
Renda familiar	Reais	Numérica contínua
Nível socioeconômico	Classificação ANEP	Categórica ordinal
Cor da pele	Observação do entrevistador	Categórica nominal
Nível de atividade física	Minutos de atividade física por aos 11 anos	Numérica contínua
Composição corporal aos 11 anos	Espessura da dobra cutânea tricipital e subescapular	Numérica contínua

7.6. Logística

No primeiro semestre de 2006, os 655 adolescentes que foram amostrados em todos os acompanhamentos anteriores da coorte serão procurados por uma equipe de pesquisa para uma visita domiciliar. No último acompanhamento da coorte (ano de 2004-5), 568 destes adolescentes foram localizados, portanto o número real de adolescentes esperado para este projeto é de 568. Nesta visita, todos responderão a um questionário com ênfase sobre dieta e prática de atividades físicas (Anexo I). Além disso, a composição corporal

será avaliada através de diluição de deutério e medidas antropométricas. As fichas que serão utilizadas para auxílio na coleta destes dados encontram-se nos Anexos I e II. A atividade física será mensurada de forma objetiva através de acelerômetros da marca Actigraph (modelo GT1M) e Actihearts em todos os adolescentes. Uma sub-amostra de aproximadamente 30 adolescentes também utilizará o acelerômetro da marca Minimitter (modelo Actical). Nesta mesma sub-amostra, o gasto energético será avaliado pelo método de água duplamente marcada. Em todos os adolescentes a maturação sexual será mensurada pelos estágios de Tanner.

Os acelerômetros serão usados concomitantemente, presos à cintura do adolescente por quatro dias consecutivos (quinta à domingo), conforme outros estudos. Nestes mesmos dias também serão utilizados os aparelhos Actihearts, sendo que este aparelho será fixado no tronco do adolescente (Brage et al., 2005).

Oito entrevistadores serão responsáveis por avaliar cerca de 30 adolescentes por semana e a duração prevista para o trabalho de campo é de cinco meses. Os entrevistadores passarão por um treinamento para correta aplicação dos instrumentos e técnicas de medidas. As atividades que os entrevistadores desenvolverão durante a visitar domiciliar são (em ordem):

- 1 – Aplicação do questionário;
- 2 – Medida da altura;
- 3 – Medida da massa corporal;
- 4 – Medida de dobras cutâneas tricipital e subescapular;
- 5 – Perímetro da cintura, quadril, braquial e da coxa;
- 6 – Coleta da saliva pré-dose (referente ao deutério);
- 7 – Aplicação da dose de deutério e anotar o endereço de onde o adolescente estará 4-5 horas depois;
- 8 – Anotar o horário da aplicação da dose;

9 – Aplicação e explicação do funcionamento dos aparelhos para verificação do nível de atividade física;

10 – Voltar no domicílio 4-5 horas após a coleta da primeira dose de saliva e coletar a amostra pós-dose de saliva (referente ao deutério)

11 – Anotar a quantidade (em número de copos, por exemplo) e tipo de líquidos ingeridos durante o período entre doses;

12 – Ainda no mesmo dia, entregar no Centro de Pesquisas as amostras de saliva e a garrafa que continha deutério. O transporte das amostras de saliva deve sempre ser realizado em uma bolsa térmica;

13 – Voltar no quinto dia após a primeira visita para recolher os aparelhos de mensuração de atividade física (acelerômetros e actihearts);

14 – Entregar os aparelhos no Centro de Pesquisas Epidemiológicas.

É de suma importância certificar-se que nos 10 minutos anteriores às coletas de saliva o adolescente não tenha ingerido absolutamente nada, nem mesmo escovado os dentes, caso contrário deve-se aguardar pelo menos 10 minutos.

Em uma sub-amostra de 30 adolescentes, ainda haverá a mensuração do nível de atividade física pelo acelerômetro Actical e gasto energético pelo método de água duplamente marcada. Nesta sub-amostra, além do questionário Block, também será aplicado um questionário de freqüência alimentar, a fim de testar a validade do instrumento. O protocolo da água duplamente marcada necessita uma amostra de urina pré-dose e pós-dose nos dias 1, 3, 10, 11 e 12. A coleta de saliva 4-5 horas após administração da dose também é necessária. A amostra de urina pode ser coletada em qualquer horário do dia, contando que não seja a primeira do dia. A quantidade de urina deverá encher cerca de 75% do tubo coletores.

Haverá uma pessoa responsável pela preparação das doses de deutério e água duplamente marcada, assim como estocagem das doses de saliva e urina. Esta pessoa possui Bacharelado em Química e experiência em técnicas laboratoriais. As dependências do Laboratório da Faculdade de Medicina da UFPel serão utilizadas para estas atividades.

Os dados armazenados nos aparelhos de mensuração do nível de atividade física serão transferidos semanalmente para um computador pelo investigador principal do estudo.

As amostras de saliva e urina serão estocadas em tubos identificados e uma vez por mês serão enviadas para Londres, onde o Prof. Dr. Jonathan Wells será responsável pela análise dos materiais.

7.7. Instrumentos

Um questionário previamente testado será aplicado aos adolescentes a fim de investigar-se hábitos alimentares e prática de atividades física (Anexo I). Também serão utilizados acelerômetros das marca Actigraph (modelo GT1M), Mini-Mitter (modelo Actical) e unidades do aparelho ActiHeart.

Água duplamente marcada e deutério serão utilizados para determinar o gasto energético diário e a quantidade de água corporal total, respectivamente. A análise destes materiais será realizada no Instituto de Saúde da Criança em Londres, sob coordenação do Prof. Dr. Jonathan Wells.

A água corporal total será determinada pela diluição de deutério, utilizando-se uma dose de aproximadamente 0,5g de óxido-deutério por quilograma de peso corporal. Amostras de saliva pré-dose e 5h pós-dose serão tomadas através de coletores de saliva de algodão.

Para avaliação da composição corporal através de outros protocolos, as seguintes medidas serão realizadas:

- peso e altura (balanças Seca e estadiômetro da UFPel)
- perímetro da cintura (Fita métrica Gulick)
- perímetro do quadril (Fita métrica Gulick)
- perímetro da porção medial do braço (Fita métrica Gulick)
- perímetro da porção medial da coxa (Fita métrica Gulick)
- dobra cutânea subescapular (plicômetro científico - Cescorf)
- dobra cutânea tricipital (plicômetro científico - Cescorf)

7.8. Entrevistadores

Oito entrevistadoras realizarão o trabalho de campo. Antes do início da coleta dos dados, os entrevistadores passarão por um treinamento teórico-prático com especialistas. Haverá ainda duas pessoas responsáveis apenas pela coleta de saliva pós-dose de deutério. Estas pessoas deverão coletar a amostra de saliva entre 4 e 5h após a coleta da primeira amostra.

8. ASPECTOS ÉTICOS

O presente projeto já foi aprovado pelo Comitê de Ética e Pesquisa da Faculdade de Medicina da Universidade Federal de Pelotas. Os dados pessoais dos participantes da pesquisa serão mantidos em sigilo. Será ainda requerido consentimento por escrito dos pais ou responsáveis para realização das medidas e entrevistas com os adolescentes.

9. ORÇAMENTO

Este estudo obteve financiamento do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ) e Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul (FAPERGS). As análises envolvendo a utilização de deutério e água duplamente marcada serão realizadas sem custos pelo Prof. Dr. Jonathan Wells, do *Institute of Child Health* em Londres.

10. CRONOGRAMA

Atividades	2006												2007											
	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	
Revisão de literatura																								
Processo de amostragem																								
Seleção e treinamento dos avaliadores																								
Estudo Piloto																								
Coleta de dados																								
Limpeza dos dados																								
Digitação																								
Análise dos dados																								
Redação dos artigos																								
Defesa da tese																								

11. DIVULGAÇÃO DOS RESULTADOS

As formas de divulgação dos resultados do estudo serão: a) artigos para publicação em periódicos científicos; b) tese de conclusão de curso de doutorado em Epidemiologia; c) um resumo dos principais resultados do estudo, a ser divulgado na imprensa local.

A proposta para os três artigos exigidos pelo programa são:

- 1) Comparação do nível de atividade física determinado por questionário, sensores de movimento e água duplamente marcada;
- 2) Mudanças nos padrões de dieta, atividade física e composição corporal dos 11 aos 13 anos;
- 3) Obesidade central na adolescência e atividade física: uma revisão sistemática.

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RELATÓRIO DO TRABALHO DE CAMPO

O artigo 1 da tese (página 61) descreve detalhadamente o trabalho de campo. Esta seção destaca apenas os aspectos mais importantes do trabalho de campo e aborda algumas questões não apresentadas no artigo 1.

A idéia de escrever um artigo apenas sobre os aspectos metodológicos do trabalho de campo foi alimentada pela complexidade do trabalho desenvolvido. Apesar da composição corporal e atividade física serem temas amplamente estudados atualmente, desconhecemos a existência de qualquer outro estudo de base populacional que tenha mensurado todas as variáveis do presente trabalho. O Quadro 1 apresenta uma descrição destas variáveis, além dos materiais levados a campo pelas entrevistadoras para coleta de dados em um adolescente.

A organização da logística das entrevistas foi uma das tarefas que exigiu muito cuidado ao ser elaborada, diante das dificuldades da mesma. O número de sensores de movimentos disponíveis para o estudo e a necessidade de coletar uma amostra de saliva 4h após a entrevista foram aspectos ímpares deste trabalho. Por exemplo, as entrevistas não poderiam ser agendadas para após as 18h, caso contrário o retorno ao domicílio teria que ser por volta das 22h. Entre os adolescentes que estudavam no período vespertino, a entrevista era agendada para antes das 12h e a coleta de saliva 4h após a entrevista acontecia no colégio onde o mesmo estudava. Para tanto, era realizado um contato prévio com a direção da escola de modo a obter-se autorização para coleta dos dados. Em alguns casos, a direção da escola exigiu um contato com os pais (ou responsáveis) confirmando a participação do adolescente na pesquisa e a permissão para coleta dos dados.

As 24 entrevistas realizadas toda quarta-feira também não deveriam ser agendadas para um mesmo horário, porque exigiria que a coleta de saliva fosse realizada no mesmo horário para todos os participantes e apenas quatro pessoas foram contratadas para a coleta de saliva (cada um dos quatro trabalhadores era responsável pela coleta de saliva em seis adolescentes). Além disso, as entrevistas eram agendadas tendo em vista o local de moradia do adolescente, de modo que facilitasse a coleta dos dados tanto por parte das

entrevistadoras como também dos responsáveis pelas coletas de saliva. Devido ao fato de que a saliva tinha que ser coletada em horário específico, apenas pessoas que tivessem carro ou preferencialmente moto foram contratados para o trabalho de coleta de saliva.

Algumas dificuldades para execução do projeto não foram associadas diretamente ao trabalho de campo, mas sim a momentos anteriores ou posteriores à coleta dos dados. Vários materiais necessários para realização do projeto foram importados em função da indisponibilidade dos mesmos no Brasil e de seus altos custos. As amostras de saliva e urina foram exportadas para o Institute of Child Health, em Londres, onde estão sendo analisadas sob a coordenação do Dr. Jonathan Wells. Dificuldades tanto para importação quanto para exportação destes materiais também foram barreiras a serem transponíveis pela equipe.

Por exemplo, 25 unidades do acelerômetro Actigraph foram importadas diretamente do fabricante, nos EUA através do Sistema Importa Fácil Ciência, do CNPq (<http://memoria.cnpq.br/importafacil/>). Se adquiridos no Brasil, o custo deste material seria 250% maior. O Sistema Importa Fácil Ciência é destinado a pesquisadores e promete facilidade e agilidade na importação de bens destinados à pesquisa, além de menores custos na importação devido à isenção de alguns impostos. Porém, nossa experiência com o Sistema foi desgastante. Inúmeros contatos foram realizados com o CNPq de modo a tentar descobrir exatamente como proceder para utilizar o Sistema. Além disso, a importação levou meses para se concretizar, o que acarretou atraso no início da coleta dos dados.

Dificuldade semelhante foi enfrentada para a exportação das amostras de urina. A tradicional empresa DHL, que anteriormente realizava exportação de materiais biológicos, não mais realiza este tipo de serviço no Brasil. Os correios passaram então a fazer a exportação deste tipo de material, porém, os mesmos desconhecem quais são os documentos exigidos pela ANVISA assim como o preenchimento correto destes formulários. Recentemente contratamos uma empresa privada para enviar as últimas amostras de urina do sub-estudo.

Quadro 1. Material necessário para entrevistar um adolescente e medidas realizadas no momento da entrevista.

MATERIAL* NECESSÁRIO PARA ENTREVISTAR UM ADOLESCENTE	MEDIDAS REALIZADAS NO MOMENTO DA ENTREVISTA
<ul style="list-style-type: none"> - 1 folha de rosto - 1 termo de consentimento - 1 questionário - 1 envelope lacrado contendo o questionário confidencial - 1 prancheta - lápis e borracha - 2 sensores de movimento (1 actiheart + 1 GT1M) - 1 folha instrução dos acelerômetros - 6 eletrodos - 1 bolsa térmica - 1 sacola plástica contendo garrafa com água e canudo - 1 salivete - 1 balança - 1 estadiômetro - 1 plicômetro - 1 fita antropométrica - 1 antropômetro - 1 balança portátil - 1 aparelho de pressão arterial portátil 	<ul style="list-style-type: none"> Pressão arterial de repouso Freqüência cardíaca de repouso Estatura Massa corporal Circunferência braquial Circunferência da coxa Circunferência da cintura Circunferência do quadril Prega cutânea tricipital Prega cutânea subescapular

* todos os materiais eram transportados em uma mochila.

ALTERAÇÕES NO PROJETO

Algumas mudanças do projeto inicial foram necessárias. Este projeto de pesquisa foi desenvolvido em parceria com duas instituições da Inglaterra: Institute of Child Health – Londres (colaboração do Dr. Jonathan Wells) e MRC Epidemiology Unit – Cambridge (colaboração do Dr. Ulf Ekelund). A parceria com estas instituições foi fundamental para a execução deste projeto.

Os pesquisadores envolvidos, ambos com grande experiência nas áreas de composição corporal e atividade física, participaram diretamente de todas as etapas do projeto. Além disso, a primeira instituição cedeu todo o oxigênio-18 necessário para as estimativas de gasto energético e se responsabilizou pelas análises da estimativa de composição corporal e gasto energético determinados pelos métodos de diluição de deutério e água duplamente marcada, respectivamente. A segunda instituição realizou o empréstimo dos Actiheart e se responsabilizou pelas análises dos dados deste aparelho.

Entretanto, ocorreram alguns imprevistos. O espectômetro de massa, necessário para as análises da composição corporal e gasto energético, esteve danificado por mais de um ano. Sendo assim, os dados sobre composição corporal e gasto energético ainda não estão disponíveis. A previsão do Dr. Jonathan Wells é de que os resultados estejam disponíveis para o mês de setembro deste ano, já que o equipamento foi reparado. Da mesma forma, a criação do algoritmo e, consequentemente, de um software para análises dos dados do Actiheart ainda não foram finalizadas.

Portanto, alterações nos artigos previstos para a tese foram inevitáveis. A proposta inicial para os três artigos era:

- artigo 1 - Comparação do nível de atividade física determinado por questionário, sensores de movimento e água duplamente marcada;
- artigo 2 - Mudanças nos padrões de dieta, atividade física e composição corporal dos 11 aos 13 anos;
- artigo 3 - Obesidade central na adolescência e atividade física: uma revisão sistemática.

O artigo 1 passou a ser sobre a metodologia do trabalho de campo. O artigo 2 investigou a associação entre atividade física determinada pelo acelerômetro Actigraph e índices antropométricos. Embora fosse possível a realização do artigo 2 inicialmente previsto com os dados disponíveis, nós optamos pela modificação para apresentarmos um artigo utilizando uma medida mais precisa do nível de atividade física dos adolescentes (dados do acelerômetro Actigraph). Finalmente, o artigo 3 necessitou de uma discreta alteração em virtude do baixo número de trabalhos que investigaram a associação entre obesidade *central* e atividade física entre adolescentes. O tema central do artigo de revisão foi expandido para composição corporal e atividade física na adolescência.

RELATÓRIO PARA IMPRENSA

PRÁTICA DE ATIVIDADE FÍSICA PODE DIMINUIR A GORDURA LOCALIZADA NA REGIÃO ABDOMINAL DE ADOLESCENTES

O percentual de adolescentes brasileiros com excesso de peso está aumentando ao longo dos últimos anos. O excesso de peso nos adolescentes tem consequências em curto e longo prazo. Por exemplo, adolescentes acima do peso têm mais chances de desenvolver hipertensão arterial, diabetes e problemas articulares. Além disso, a obesidade desenvolvida na infância e adolescência tende a persistir na idade adulta.

Avaliar o local do corpo onde a gordura se acumula é tão ou mais importante do que avaliar a quantidade total de gordura do corpo. Estudos apontam que a gordura localizada na região do tronco (cintura) é mais prejudicial à saúde do que a gordura localizada nos braços e pernas.

A prática de atividade física normalmente é recomendada como uma das estratégias para prevenir e tratar a obesidade, porém, poucos estudos investigaram o efeito da atividade física sobre a deposição de gordura na região da cintura de adolescentes. O professor de Educação Física Felipe F Reichert, sob orientação da professora Ana Maria B Menezes conduziu uma pesquisa sobre o assunto.

Foram avaliados 457 adolescentes com idades entre 12 e 14 anos. A prática de atividade física foi avaliada por acelerômetros, que são pequenos aparelhos para medir a quantidade e a intensidade dos movimentos do corpo a cada 5 segundos. Os adolescentes utilizaram os aparelhos por 4 dias consecutivos, incluindo o final de semana. A gordura da região abdominal foi avaliada por um método simples, mas também muito preciso: a circunferência da cintura (medida com uma fita métrica).

Os resultados do estudo indicaram que a prática de atividade física pode diminuir a gordura abdominal. Porém, a intensidade da atividade é um importante fator a ser considerado. Apenas atividades de intensidade mais forte, como por exemplo, a corrida demonstrou algum benefício sobre a diminuição de gordura. O efeito das atividades de

intensidade moderada, como a caminhada, foi menos evidente. Além disso, os autores verificaram que quanto maior o tempo praticando atividades de intensidade forte, maior o benefício sobre a gordura abdominal. Porém, vale destacar também que com apenas 15 minutos diários de atividade física já são observados efeitos significativos.

Os autores ressaltam que apesar das atividades de intensidade moderada não terem demonstrado efeito sobre a composição corporal, a prática regular deste tipo de atividade, como, por exemplo, a caminhada, traz vários outros benefícios à saúde. Durante a adolescência, é importante que o jovem experimente vários tipos de atividades, independente da intensidade da mesma. Desta maneira, há mais chances do adolescente descobrir uma atividade que seja prazerosa e divertida para ele. E apenas atividades prazerosas e divertidas são praticadas regularmente por um longo período de tempo, quando, então, os inúmeros benefícios provenientes da atividade física poderão ser observados.

**A methodological model for collecting high-quality data on physical
activity in developing settings - The experience of the 1993 Pelotas
(Brazil) Birth Cohort Study**

Aceito no Journal of Physical Activity and Health

Carta de aceite

18-Jul-2008

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A methodological model for collecting high-quality data on physical activity in developing settings - The experience of the 1993 Pelotas (Brazil) Birth Cohort Study

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ABSTRACT

Background

Prospective studies on physical activity (PA), diet, and body composition in adolescents are lacking, particularly outside high-income countries. The aims of this paper were to describe the methods used for assessing these variables in the 1993 Pelotas (Brazil) Birth Cohort, and to discuss the fieldwork challenges faced and alternatives to overcome them.

Methods

In 2006-7 a sub-sample of the 1993 Pelotas cohort was revisited. PA was estimated using questionnaires, a combined heart rate and motion sensor (Actiheart), and the Actigraph GT1M accelerometer. Diet was investigated by questionnaire. Total body water was determined by stable isotopes. Thirty individuals had their total energy expenditure assessed by double labeled water. All data were collected at participants' home.

Results

The logistics of the fieldwork and the difficulties in undertaking the study and alternatives to overcome them are presented. Preliminary analyses show that 511 individuals were traced (response rate = 90.0%). Compliance of both adolescents and their families for the motion sensors and body composition measurements was excellent.

Conclusions

We conclude it is feasible to carry out high-quality studies on PA in developing countries. We hope the present manuscript will be useful to other researchers interested in carrying out similar studies.

Background

Currently, an epidemic of obesity is observed in several countries. While excess body weight may appear to be a response to affluence, studies show it's prevalence is increasing rapidly in low- and middle-income as well as developed countries ¹⁻⁴. In Brazil, the prevalence of overweight among boys aged 10 to 19 increased from 2.6% to 11.8% from 1975 to 1997; while in girls it rose from 5.8% to 15.3% in the same period ⁴. Substantially less is known about the aetiology of obesity in non-western settings, reducing the opportunities to implement appropriate public health strategies.

Although measurements of physical activity patterns, energy expenditure, diet, and body composition in epidemiological studies are essential to the understanding of the aetiology of childhood obesity, very few studies have measured these variables objectively. Most studies rely on self-reported data collected through household face-to-face interviews, as more detailed outcomes either involve non-transportable equipment or excessive labour requirements. Conversely, studies in low- and middle-income countries which try to examine subjects in clinics or medical facilities tend to present substantially lower response rates than those using household-based samples, risking epidemiological bias.

Due to these difficulties, obesity is typically measured using an outcome – body mass index – that has a poor association with body fat on an individual basis, ⁵ and risk factors are subjectively reported rather than objectively measured. However, recent advances allow a markedly more sophisticated approach to be adopted, including stable isotope probes and combined heart-rate and motion recording monitors. These technologies are ideal for community-based research in low and middle-income settings. The 1993 Pelotas (Brazil)

Birth Cohort Study constitutes a distinctive opportunity to prospectively investigate physical activity, energy expenditure, diet and body composition in a population rapidly undergoing nutritional transition. The aim of this paper is to describe the methods used for assessing such variables in a sub-sample of this cohort, and to discuss the fieldwork challenges faced and alternatives to overcome them. We hope this information will benefit others addressing the obesity epidemic in modernizing countries.

Methods/design

A detailed description of the 1993 Pelotas (Brazil) Birth Cohort Study is available elsewhere⁶. In brief, 5249 newborns delivered in the city's hospitals (>99% of all deliveries) were enrolled, and sub-samples were visited at one, three and six months, one and four years. In 2004-5, all cohorts members were sought, and 87.5% could be traced. There were 568 subjects who were studied in all follow-up visits of the cohort and revisited from August 2006 to January 2007.

In the previous visits, data on several variables, including growth pattern, socioeconomic level, religion, physical activity, and diet were gathered⁶. The present study focused on four aspects: diet, physical activity, body composition, and energy expenditure. The later was measured by double labeled water (DLW) in a sub-sample of 30 adolescents, while the remaining (diet, physical activity pattern and body composition) was measured in all cohort members eligible for this investigation. The Ethical Committee of the Federal University of Pelotas Medical School approved the study protocol and written consents were obtained from parents or guardians. Details of each variable assessed are provided below.

Recruitment, training, and selection of fieldworkers

As in previous follow-up visits, data were mostly collected in the participant's home. We invited 22 candidate interviewers who had previous experience in face-to-face interviews and anthropometric techniques with children and adolescents to undergo training sessions for correct application of the questionnaires and anthropometric measurements. All interviewer applicants were women, adults (aged 18 years or more), had at least high school degree, and were blinded in terms of objectives and hypotheses of the study. Overall, ten training sessions were performed in a week, comprising 40 hours of theoretical and practical training. The training was carried out in the facilities of the Post-graduate Program in Epidemiology of the Federal University of Pelotas. The same researchers who trained the interviewers for the 2004-05 follow-up regarding the physical activity and dietary questionnaires also trained the interviewer applicants in the present follow-up.

An experienced anthropometrist trained the interviewer applicants with a group of subjects of comparable age to the cohort members. In addition to the training sessions in the University's facilities, the skinfold measurements were also practiced in a public school. Ten boys and girls born in 1992 participated in the training. We chose to select slightly older students so that there would be no risk of selecting a cohort member for training purposes. Because this part of the training was performed during adolescents' school time, and therefore, time was a constraint, only the skinfolds were measured. Interviewer applicants who did not achieve the criteria of the NCHS (National Center for Health Statistics, United States) for intra- and inter-interviewer errors were eliminated (n=6). Based on the performance in the training, 12 interviewers were selected to carry out the interviews.

In addition to the 12 interviewers working in this project, there were four fieldworkers who were responsible for collecting saliva samples and accelerometers from the participants' homes; three graduate students whose jobs included organizing the material for the interviewers, reviewing questionnaires, and scheduling interviews, one chemist who was responsible for preparing the deuterium and DLW doses and one fieldwork supervisor. Throughout the text, the term "fieldwork supervisor" will be used to with reference to the activities developed by the graduate students.

Physical activity

Physical activity is a multi-dimensional variable, incorporating a spectrum of outcomes including daily schedule, frequency, duration, intensity, cardiac output and energy cost. Questionnaires are good at assessing some of these dimensions, but are poor for discerning others, especially intensity and energy cost. Assessment of physical activities through questionnaire is even more challenging in the adolescent population, because the dynamic nature of the activities of this group. In the present study, physical activity was measured by both subjective (questionnaire) and objective (accelerometers) methods.

Questionnaire

Physical activity was estimated through a pre-tested and standardized questionnaire. This questionnaire has been used in previous studies with this cohort^{7,8}. The questionnaire investigated physical activities related to the mode of transportation to and from school, physical activities inside and outside school settings, as well as leisure-time activities. Data

on time spent in sedentary activities, like playing video-game, using the computer or watching TV were also collected.

Accelerometers

The Actigraph accelerometer (model GT1M) and the Actiheart, which is a combined heart rate and movement sensor were also used to determine physical activity level. Technical details of these devices as well as data on their validity are available elsewhere⁹⁻¹¹. The Actigraph was placed on the left side of the waist, while the Actiheart was attached to the chest with two standard electrodes, according to the manufacturer's instructions. Because the electrodes lose their ability to adhere to the skin surface over time, extra electrodes were made available for the subjects and they were instructed on how to replace the electrodes, if necessary. In addition, an instruction sheet for the accelerometers, which contained a brief description of the devices, details of how to wear them, and contact information was left at the participant's home at the time of the interview. This instruction sheet also had a diary for the devices. Participants were instructed to note if they did not wear one or both of the monitors for any period >1 hour during the day.

Subjects wore the monitors from Wednesday to Monday and they were encouraged to wear them 24 hours per day, except when showering, bathing, or swimming. Usually on Monday morning, fieldworkers visited the participant's home to collect the monitors and the diary which provided any notes regarding the use of the devices (for example, if the adolescent did not wear any of the devices for more than 1h, he/she was instructed to describe it in the diary).

On Monday afternoon/evening, the monitors were downloaded and the devices were placed on battery chargers. On Tuesday the recharged monitors were set up for new cohort members.

Body composition

Body composition concerns fat and lean tissue masses, which are related to body size and physique. Most research on body composition has used indices of size (weight and height, combined as body mass index, BMI), as the primary index of adiposity. However, BMI is a poor index of fatness in individual children,⁵ and may identify spurious associations between risk factors and outcomes. For example, increased BMI may be due to greater fat mass or lean mass: whereas exercise is predicted to decrease fatness, it is predicted to increase lean mass. The comprehensive relationship between physical activity practice and body composition is therefore unlikely to be discerned using BMI. Other anthropometric indices such as skinfold thicknesses are good for discerning variability in specific fat depots, but unreliable for estimating either total body fat mass, or lean mass. Anthropometry is important for assessing physique and regional fat distribution, but tissue masses require a more accurate approach.

Anthropometry

Body weight was measured to the nearest 0.1kg with participants wearing light clothing without shoes by the Tanita scale model BF-680W. Height was determined to the nearest 0.1cm using stadiometers from the Medical School of the Federal University of Pelotas. Tricipital and subescapular skinfolds were measured to the nearest 0.1mm using the Scientific Cescorf® calipers and followed the recommendations of Lohman¹². Three

measures of each site were taken. We also measured the following circumferences: waist (thinnest circumference), hip (largest circumference), middle thigh, and middle arm. Circumferences were taken using a flexible steel metric tape to the nearest 0.01cm.

Stable isotopes

Deuterium dilution is the most accurate field method for investigating body water in children ¹³. It is ideal for large-scale studies, being non-radioactive and non-invasive and requiring minimal effort from the subject. Doses of deuterium dilution were prepared by a chemist every Monday. Bottled mineral water was used to make doses up to required level. Due to the fact that doses were prepared prior to the interview and current weight of the individual was unknown at that time, a fixed amount of deuterium (2.2g) was employed. At the end of the dose preparation, a sample of its content was transferred into a 2ml labeled microtube and stored in a freezer. The bottle was put into a plastic bag with a drinking straw, and the bag weighed before and after dose administration.

Saliva samples were collected before and 4 (normal body mass index for age) or 5 hours (overweight or obese individuals) after administration of the deuterium dose. Individuals were asked to provide the place where they would be 4h (or 5h) after dose administration, so that the second saliva sample could be collected. In many cases, individuals would not be at home and therefore the post-dose saliva sample had to be collected in several different settings.

Participants took the cotton wool from salivette and returned it afterwards, avoiding need for gloves. The cotton wool was left in the mouth for 1 to 2 minutes. Fieldworkers were instructed to ensure that no liquid had entered the mouth in the prior 10 minutes (i.e. no drinks, mouth-rinsing or teeth-cleaning). The salivette was transferred to the research centre

in cool-bags, avoiding excessive heat. Pre- and post-dose saliva samples were centrifuged separately at 6.000 rpm for 5 minutes and thereafter transferred into 2ml microtubes to be stored in the freezer. A Marte scale (model AS1000C) was used to prepare the doses and the Fanem (model 208N) centrifugal machine was used to centrifuge the salivettes.

Diet

The Block questionnaire¹⁴ was applied to all participants. This questionnaire investigates consumption of fat and fiber in the last year. It is a food-frequency questionnaire and has a self-score. As the physical activity questionnaire, this dietary questionnaire was also applied in the 2004-05 follow-up, therefore, we were able to investigate tracking of these variables.

Energy expenditure sub-study

We randomly selected 10 individuals from each tertile of socioeconomic status as in the 2004-05 follow-up, for determination of energy expenditure by DLW. Dose preparations were made by the same chemist responsible for preparing the deuterium doses. Doses were prepared using 1.25g/kg of 10% 18-oxygen labeled water and 0.075g/kg of 99.9% deuterium labeled water. Pre- and post-dose saliva samples were collected as described in the deuterium measurement. Additionally, urine samples were collected pre-dose and one, two, three, eight, nine, and 10 days after dose administration. Fieldworkers visited participants' home every day of data collection in order to make sure the participants had collected the urine sample. Urine samples were kept in freezer until posting to the UK for analysis.

Logistics of the fieldwork

Table 1 shows an overview of selected day-by-day tasks performed during the period of data collection. We aimed to measure 24 individuals per week (two interviews were assigned to each one of the 12 interviewers). The interviews were previously scheduled with the adolescent's parents or caretakers through phone calls. Four fieldworkers were responsible for collecting the post-dose saliva sample (each one was responsible for 6 adolescents). Motorcyclists were preferred for this task because this mode of transportation allowed easier arrival in the participants' home at the required time. These four fieldworkers were also responsible for collecting the accelerometers on Monday in the participants' homes. The interviews were scheduled taking into account the location of the participants' homes.

Both interviewers and motorcycle drivers had a cool bag for storage of saliva samples. These materials were brought to the laboratory on Thursday morning and the saliva samples were centrifuged upon their arrival.

On Thursday and Friday new cohort members were contacted for scheduling the visit. On Fridays fieldwork supervisors also performed quality control. Quality control was conducted by phone calls in 50% of the sample (one interview/week randomly selected from each interviewer). Supervisors were instructed to talk with either the adolescent or their parents and ask about the experience provided by the interviewer and the anthropometric measures taken, and to repeat some questions of the questionnaire.

On Mondays data from all the 48 devices used in the study (24 Actigraphs and 24 Actihearts) were downloaded and the devices were immediately put into the charger so that they could be set up for new users.

Preliminary results

Figure 1 presents an overview of the main results of the fieldwork. Each interview lasted approximately 45min. The response rate was 90.0%. The main reason for loss to follow up was that many cohort members had moved away from the city. Only 15 individuals refused to participate in the study (8 boys).

Initial analyses showed that 498 individuals wore the Actigraph accelerometer and 457 individuals (238 boys) wore it for at least 600min/day. The overall registered time showed an average of 921min/day ($sd = 92$) and a range of 620 to 1266min/day. The mean time spent in moderate (2000 to 4999 counts per minute (cpm)) and vigorous-intensity activities (≥ 5000 cpm) was 63 and 9 min/day, respectively. Box 1 shows further results regarding the use of the Actigraph and physical activity data for boys and girls. Equivalent analyses for the Actiheart monitor have not yet been made.

Discussion

Prospective data on the inter-relationship between physical activity, diet, and body composition in adolescents are lacking, particularly in low- and middle-income countries. In this context, the 1993 Pelotas (Brazil) Birth Cohort Study constitutes an exceptional opportunity to investigate this relationship. The present study described the methodology used to assess these variables in a sub-sample of the 1993 Pelotas (Brazil) Birth Cohort.

One of the novelties of the present work is the simultaneous utilization of two different motion sensors to estimate physical activity. The use of motion sensors is becoming increasingly common, particularly in studies with children and adolescents. In spite of several advantages, motion sensors depend on the compliance of the subjects (i.e. subjects must wear it) an issue that might concern other researchers. Preliminary analyses of our data show that out of the 511 individuals measured, 457 individuals wore the accelerometers for at least 10 hours/day. Furthermore, 93.2% of individuals wore it for three or more days, indicating high acceptance of the devices by the adolescents. It should be noted that several strategies were adopted to achieve this high rate, including: a) at the moment of the post-dose saliva sample (4 to 5h pos-interview) the fieldworker was instructed to check whether the individual was wearing the devices and to ask the adolescents if he/she had any concerns regarding the devices; b) two days after the interview, individuals were called for quality-control purposes and were again questioned about any concerns regarding the devices, and encouraged to keep on wearing them; c) at the moment of the interview a comprehensive sheet of paper which contained detailed information about both of the devices and telephone numbers so that they could call at any time in case of doubts was left in the participants' home. We used 24 units of each type of motion sensor per week to collect the data. None of the devices were lost but two units of the Actigraph and approximately eight Actihearts were replaced due to malfunction.

The high number of adolescents measured by stable isotopes in the present study should also be highlighted. Stable isotopes are considered the most accurate method to estimate body water ¹⁵. Most large-scale studies do not measure body composition, and rely only on

simple indices of obesity as body mass index. Some practical drawbacks of the deuterium dilution method should be noted, however. First, some parents demonstrated some concern with the fact that their offspring were asked to ingest the deuterium dose (potential collateral effects). We believe that this issue might be faced by other researchers conducting population based studies in low- and middle-income countries. In order to address this drawback the interviewers were strongly trained to explain the purpose of the dose administration. Both isotopes used are naturally occurring and non-radioactive, and there are no known health risk associated with these measurements. Another alternative to overcome this barrier would be to ask the subjects to go to a research center, where data collection (including deuterium dose administration) would take place. Nonetheless, it is likely that a much lower response-rate would be achieved by this later strategy, risking selection bias. Finally, although at the moment of the interview individuals were asked to inform where they would be 4 to 5 hours after dose administration (to collect the second saliva sample), some of them could not be located. In these cases adolescents were contacted to re-schedule the measurements.

The number of measurements taken at participants' homes in this study should also be noted. These measurements included body weight, height, two skinfold thickness, four circumferences, application of questionnaires, and collection of several saliva and urine samples (this later in a subsample only). Furthermore, adolescents were asked to wear two motion sensors for nearly five days continuously. This is a unique aspect of this follow-up since previous visits to this cohort relied mostly on data collected from questionnaires and few objectively measured variables (usually only body weight, height and skinfold thickness). The response-rates for the earlier cohort visits have been very high⁶ and we

hypothesize that this might be partially attributable to the low burden of the participants.

We are not sure if the present follow up will impact negatively on the response rate of future visits to the cohort. Nonetheless, researchers dealing with cohort studies must be concerned about this issue because loss to follow up is a major drawback of cohort studies and may seriously bias the results.

In summary, this paper describes logistical and practical issues of a longitudinal study investigating diet, physical activity, energy expenditure, and body composition in adolescents. The high response rate achieved, even on variables that require great compliance from the individuals (i.e., wearing an accelerometer for days), highlight the methodological quality of the study. We hope that the present article may be useful to other researchers interested in carrying out similar studies in other low or middle-income settings.

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Table 1. Description of a typical week of data collection. 1993 Pelotas (Brazil) Birth Cohort Study.

	Monday	Tuesday	Wednesday	Thursday	Friday
Morning	Collect the accelerometers in the adolescents' home Prepare deuterium doses	Prepare and revise the materials for the interviewers (questionnaires; weight scales, salivettes, skinfold calipers, etc.)	Interviews take place	Receive material from interviewers Centrifuge the salivettes	Perform quality control Call new cohort members to set next week's interviews
Afternoon	Download and recharge the accelerometers Prepare deuterium doses	Hand over material for the interviewers	Interviews take place	Centrifuge the salivettes Store the saliva samples Call new cohort members to schedule next week's interviews	Perform quality control Allocate next week's interviews among interviewers
Evening	Set up the accelerometers for new users		Interviews take place		

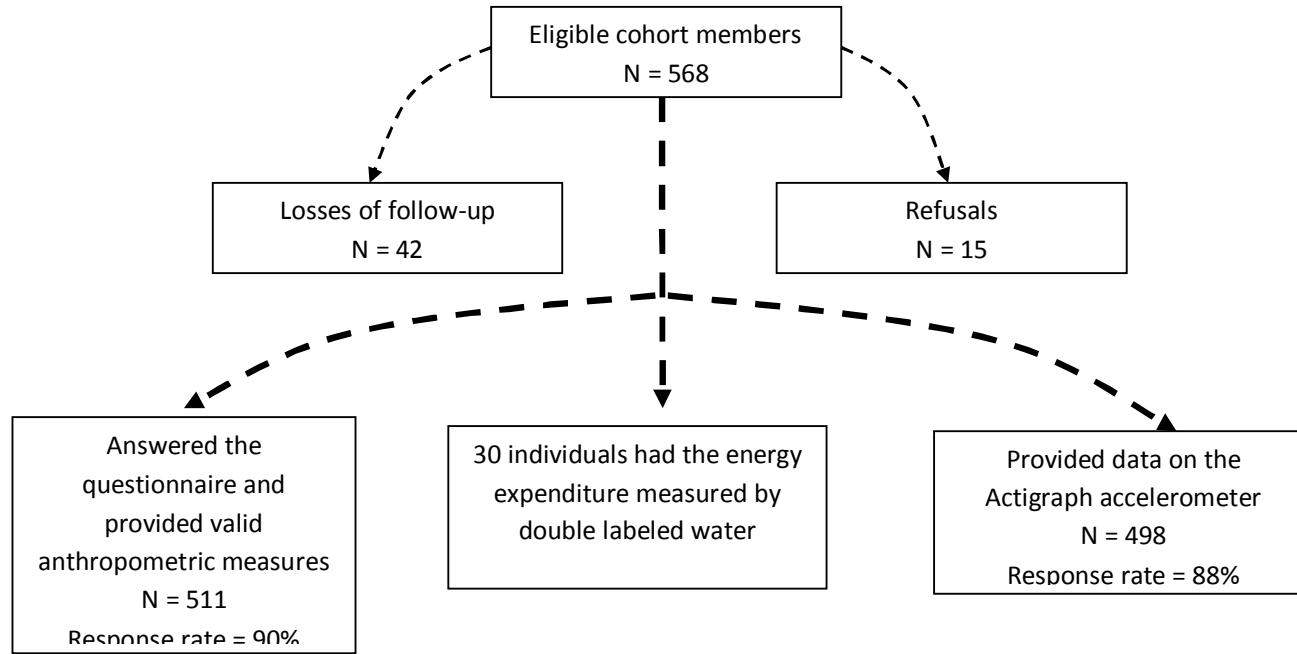


Figure 1. Preliminary results of the study. 1993 Pelotas (Brazil) Birth Cohort Study.

Box 1. Descriptive physical activity data according to the Actigraph data

	Boys N (%)	Girls N (%)
Number of days which the Actigraph had registered time above 600min		
1	2 (0.8%)	11 (5.0%)
2	9 (3.8%)	9 (4.1%)
3	27 (11.3%)	33 (15.1%)
4	101 (42.4%)	90 (41.1%)
5	94 (39.5%)	72 (32.9%)
6	5 (2.1%)	4 (1.8%)
Time spent in moderate-intensity activities (2000 to 4999 cpm)		
0 – 10min/day	3 (1.3%)	5 (2.3%)
11 – 20min/day	14 (5.9%)	22 (10.1%)
21 – 30min/day	21 (8.8%)	36 (16.4%)
31 – 40min/day	33 (13.9%)	31 (14.2%)
41 – 50min/day	34 (14.3%)	38 (17.4%)
51 – 60min/day	32 (13.5%)	28 (12.8%)
61 – 70min/day	29 (12.2%)	23 (10.5%)
71 – 80min/day	23 (9.7%)	15 (6.9%)
>= 81min/day	49 (20.6%)	21 (9.6%)
Time spent in vigorous-intensity activities (>=5000 cpm)		
0 – 10min/day	136 (57.1%)	183 (83.6%)
11 – 20min/day	72 (30.3%)	29 (13.2%)
21 – 30min/day	23 (9.7%)	7 (3.2%)
31 – 40min/day	4 (1.7%)	0 (0%)
41 – 50min/day	3 (1.3%)	0 (0%)

cpm = counts per minute

Physical activity and body composition indices in Brazilian adolescents

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Running head: Physical activity and adiposity in adolescents

ABSTRACT

Objective: To explore the association between physical activity measured by accelerometers and body composition indices in a sample of adolescents living in a population undergoing rapid nutritional transition.

5 **Design:** Population-based cross-sectional study nested to the 1993 Pelotas (Brazil) Birth Cohort.

Subjects: 457 boys and girls (80.5% of the target sample, 52.1% boys).

Measurements: Physical activity was measured by the Actigraph accelerometer. The outcomes investigated were sum of triceps and subscapular skinfolds, waist circumference, 10 waist-to-hip ratio and waist-to-thigh ratio.

Results: After adjustment for sex, socioeconomic status, age, Tanner stage of sexual maturation, diet pattern, height, and birth weight, time (min/day) spent at vigorous intensity activity was inversely associated with waist circumference and sum of skinfolds. A one minute increase in vigorous intensity physical activity was associated with a reduction of 15 0.15cm in waist circumference ($p=0.007$) and 0.20mm in sum of skinfold thicknesses ($p=0.02$). Associations were stronger in boys than in girls. Total activity and time spent in moderate intensity physical activities were not associated with any of the outcomes in the adjusted analyses.

Conclusion: Promotion of improvements in body composition in adolescents should focus on 20 vigorous intensity, instead of moderate intensity physical activities.

Keywords: Brazil, accelerometry, adiposity, body fat, motor activity, activity monitors.

INTRODUCTION

Adolescent obesity has increased not only in developed societies, but also in some low- and middle-income countries. In Brazil the prevalence of overweight adolescents increased from 5 2.6% to 11.8% among boys and from 5.8% to 15.3% among girls during the period from 1975 to 1997 (1). The consequences of total and central-fat accumulation have been well described (2). Furthermore, obesity developed in infancy and adolescence tracks into adulthood (2, 3).

Fat accumulation is ultimately a result of chronic positive energy balance. Therefore, physical 10 activity energy expenditure may play a key role. Surprisingly the literature on this subject is conflicting, as some observational (4, 5) and even experimental studies (6) showed no effects of physical activity on body composition of adolescents. Recent reviews have identified a number of shortcomings in the literature which might contribute to the inconsistency among studies (7, 8). In particular, significant limitations derive from the poor accuracy of physical 15 activity and body composition measurements.

Regarding body composition, body mass index (BMI) is the most frequent outcome used in large-scale studies (8). Although this index is widely used in pediatric epidemiological research, due to the ease with which the required measurements of weight and height can be 20 acquired in large samples, it has been shown that for a given BMI, a wide range of fat mass may be observed (9), limiting the validity of BMI as a body composition measure. Furthermore, the association between BMI and physical activity may be difficult to evaluate because of the antagonistic effects of physical activity on fat mass and fat-free mass. Other simple anthropometric measures such as circumferences or skinfolds taken at specific 25 anatomical points may provide more precise information on body composition. For example,

waist circumference is highly correlated with abdominal fat determined by magnetic resonance imaging in adolescents (10, 11).

Accurate measurement of physical activity level in adolescents is also challenging. To date,
5 most data on associations between physical activity and body composition come from physical activity measured by questionnaires (12). However, many adolescents are likely to be unable to accurately report their physical activity level. More recently, data from objectively measured physical activity (i.e. motion sensors) have emerged and results from these studies are important because they are less prone to measurement error. Only a few
10 studies have investigated the association between physical activity measured by accelerometer and body composition (13-15), and these studies have been conducted in western industrialized settings, where the majority of physical activity comprises leisure-time exercise or school-organized physical education. In summary these studies show an inverse association between physical activity level and body fat indicators, which might be intensity-dependent,
15 however the association between different sub-components of physical activity (i.e. time spent sedentary, at light, moderate and vigorous intensity activity) with body fat in adolescents from less economically developed populations remains unclear. The answer to this is important for preventive action and for public health purposes when establishing evidence-based physical activity recommendations aimed to improve body composition.

20

Therefore, the aim of the present study was to explore the association between physical activity measured by accelerometers and body composition indices in a population-based sample of adolescents living in an area rapidly undergoing nutritional transition.

25

METHODS

Study design and participants

The present study is a cross-sectional study nested within the 1993 Pelotas (Brazil) Birth Cohort. Data collection was carried out from August 2006 to January 2007. A detailed description of the cohort, its design, methods and measurements taken at each follow up until 2004 is available elsewhere (16) and another recent publication has focused on the methodological aspects of the present follow up (17). In brief, all 5249 newborns delivered in Pelotas' hospitals in 1993 constitute the cohort and sub-samples of the cohort were reexamined at age one, three and six months and at one and four years. Hospital births comprised more than 99% of all births in the city in 1993. In 2004, all individuals belonging to the cohort were eligible for another follow up and 87.5% could be traced. Five hundred and sixty eight boys and girls who were examined at all five previous follow ups comprise the target sample of the present study. These individuals show no substantial differences from the original cohort in terms of sex ($p=0.26$) and birth weight (mean difference 66g), although the difference regarding birth weight is statistically significant ($p=0.01$). Compared to data from the 2004 follow up, these individuals had similar prevalence of self-reported physical inactivity (<300min/wk of moderate to vigorous intensity activity) but were slightly more overweight than the remaining cohort members (25.2% versus 23.2%; $p=0.005$). The Ethical Committee of the Federal University of Pelotas Medical School approved the study protocol and written informed consent was obtained from parents or guardians

Anthropometric measures

Triceps and subscapular skinfolds were measured to the nearest 0.1mm using the Scientific Cescorf® calipers following the recommendations of Lohman (18). Three measurements were

taken at each site and the average of these was calculated and used in analyses. Waist circumference was measured at the thinnest circumference midway between the lower rib margin and iliac crest; hip circumference was measured at the maximal circumference over the buttocks, and thigh circumference at the mid-point between the iliac crest and patella. All 5 circumferences were taken using a flexible steel metric tape to the nearest 0.5cm. Data were collected at the adolescent's home by research assistants specifically trained by a highly experienced anthropometrist. All research assistants achieved the criteria of the National Center for Health Statistics (United States) for intra- (maximal error allowed = 1.83 and 0.8 for subescapular and tricipital skinfolds, respectively) and inter-interviewer (maximal error 10 allowed = 1.53 and 1.89 for subescapular and tricipital skinfolds, respectively) skinfold measurement errors (19) and had previous experience in face-to-face interviews with adolescents. During the training sessions, ten adolescents of comparable age to the cohort members were measured. The mean intra-and inter-interviewer errors for the skinfold measurements in these subjects were 0.355mm ($sd=0.173$) and 0.604mm ($sd=0.154$).

15

Outcomes

Based on the anthropometric variables the following outcomes were included in our analyses: waist circumference (cm), sum of triceps and subscapular skinfolds (mm), and waist-to-hip and waist-to-thigh ratios (18). Whereas a large waist circumference increases cardiovascular 20 risk (20), having an increased thigh circumference has been shown to be protective against type 2 diabetes and cardiovascular disease (21, 22). Combining information from these two body regions, sagittal diameter adjusted for thigh girth appears most strongly associated with cardiovascular risk (23).

25

Physical activity assessment

Free-living physical activity was assessed with the Actigraph accelerometer (model GT1M, Actigraph Inc, Fl, US) for 5 consecutive days (Wednesday to Monday). Output from this motion sensor is significantly correlated with physical activity energy expenditure measured during free-living conditions by the doubly labeled water method in children and adolescents (24, 25). Activity data from the accelerometer were recorded every 5 seconds. A customised program (MAHUFFe; www.mrc-epid.cam.ac.uk) was used for analysis of the Actigraph data. Only days on which >600min of data were included in further analyses. The physical activity variables investigated were total counts divided by registered time (counts per minute (cpm)), time (min/day) spent at sedentary activities (<100cpm), time spent at light intensity activities (100-2000cpm), time spent at moderate intensity physical activity (MPA, 2001-3999 cpm) and time spent at vigorous intensity physical activity (VPA, ≥ 4000 cpm). This intensity classification is in accordance with previous studies (13, 26).

15 Potential confounding factors

Several factors were considered as potential confounders of the associations under study.

These factors are described below:

Diet

20 Consumption of fibers and fat was investigated by the Block food frequency questionnaire (27). This questionnaire collects information on the consumption of 15 fat-rich (hamburgers, beef steaks/roasts, fried chicken, hot-dog, ham, mayonnaise, margarine/butter, eggs, bacon, cheese, integral milk, fried potatoes, pop-corn, ice-cream, bread/rolls/ crackers) and 12 fiber-rich nutrients (orange juice, fruits, salad, potato (not fried), dried beans, other vegetables,

cereals, grain-bread, white bread, soda, diet soda, candies/dessert) in the last year. It is a food-frequency questionnaire and has a self-score.

Socioeconomic status

5 Socioeconomic status was estimated at the 2004 follow up according to the Brazilian Criterion of Economic Classification (28). This classification takes into account the number of specific household assets, presence of domestic servants and education of the head of the family. Individuals were categorized into one of five categories, from A (wealthiest) to E.

10 **Sexual maturity**

Sexual maturity was investigated through a self-reported confidential questionnaire. The images representing the different Tanner's stage of maturation were presented to the adolescent and he/she was instructed to mark the one that most closely matched with his/her appearance. This strategy has been used in other epidemiological studies with no evidence of bias (29). Pubertal stage was based on the amount of pubic hair and stage of genital development. Girls were also questioned whether they already had attained menarche or not.

Height

20 Skinfolds and waist circumference are correlated with height (30), so we included this variable as a potential confounding factor in the multivariable models. Height was measured without shoes to the nearest 0.1cm using a portable stadiometer.

Birth weight

Low birth weight has been linked with adverse health outcomes in adolescence, including 25 both lean mass and abdominal adiposity (31). Therefore, we considered birth weight as a

possible confounder of the association between physical activity and body composition. Birth weight was measured by a trained research assistant in the hospitals using a calibrated scale within a few hours after delivery. This strategy was chosen because bias from the hospitals' staff measurements was a concern.

5

Statistics

Differences between boys and girls were tested using T tests for continuous variables and qui-square tests for categorical ones.

Simple and multiple linear regressions for the whole sample and stratified by sex were run to

- 10 test the association between sub-components of physical activity (accelerometers total cpm; time spent in MPA and time spent in VPA) and body composition variables (outcomes).

Three multivariable models were built for the whole sample analysis. The first one included only socioeconomic status as a covariate, while the second model included only sex. The third model included socioeconomic status, sex, age, Tanner stage of sexual maturation, diet pattern (i.e. fat and fibers scores), height, and birth weight. In the sex-stratified analyses, the multivariable model included all variables of the third model (except sex).

We also calculated the average of waist circumference and sum of skinfold thickness according to quintiles of minutes per day spent in VPA. The percentage of individuals who practiced $\geq 60\text{min/day}$ of at least moderate intensity activities is described.

- 20 All analyses were performed using Stata 9.2 and a p value < 0.05 was considered as statistically significant.

RESULTS

Out of the 568 individuals eligible to participate in the study, 511 were interviewed and 457

- 25 (80.5% of the target sample, 52.1% boys) provided valid data on physical activity. Over 90%

of individuals were the accelerometer for two or more days. Individuals who did not provide valid physical activity data did not differ significantly from the remaining sample in terms of current weight ($p=0.42$), height ($p=0.79$), and sum of triceps and subscapular skinfolds ($p=0.52$). Table 1 shows a description of the sample in terms of socio-demographic, sexual maturity, and dietary variables. Most of participants were aged 13 years (84.3%), while the remaining were aged 12 years (15.1%) or 14 years (0.6%). The distribution of socioeconomic status of the individuals was as follows: categories A and B (highest groups) = 21.5%; category C = 34.9% and categories D and E = 43.6%. Over one fifth (23.2%) of the girls reported not having attained menarche, and over 65% reported being in Tanner's pubertal stages IV or V. Almost 50% of boys reported being in Tanner stages IV and V. Table 2 shows a description of the sample regarding the physical activity and body composition variables. Accumulated time (min/day) spent at MPA and VPA was significantly higher in boys than girls. In contrast, boys had lower values of skinfolds in comparison to girls. Waist circumference and waist-to-hip and waist-to-thigh ratios were higher in boys than girls.

15

Crude and adjusted regression coefficients for the associations between physical activity variables with body composition outcomes are shown in Table 3 (whole sample) and Table 4 (stratified by gender). Overall, VPA were more strongly associated with the outcomes than MPA. Table 3 shows that vigorous intensity physical activity was significantly and inversely associated with waist circumference and sum of skinfolds in both crude and adjusted analyses, whereas moderate intensity activity and accelerometer counts/minute were not associated with any of the outcomes in the adjusted analyses (models 3). A one minute increase in vigorous intensity physical activity was associated with a reduction of 0.15cm in waist circumference. The association between sum of skinfolds and vigorous intensity physical activity was attenuated after adjustment for the potential confounders (mainly due to the sex variable).

Further, all physical activity variables were significantly and directly associated with waist-to-hip and waist-to-thigh ratios in the crude analyses, but not after adjustments for confounding factors. Time spent at sedentary or at light intensity activity were not significantly associated with any of the outcomes in crude or adjusted analyses. No significant interaction between 5 physical activity variables with sex was observed for any of our analyses.

In the gender-stratified analyses (Table 4), most of the associations were non-significant. In fact, none of the crude or adjusted analyses were statistically significant for girls. In boys, the time spent in vigorous activity was significantly and inversely associated with waist 10 circumference and skinfolds in the crude analyses. However, the association between waist circumference and vigorous activities was no longer significant ($p=0.17$) in the adjusted analyses, whereas the association between vigorous intensity activity with skinfold thicknesses presented a p value of 0.14.

15 Figure 1 shows the mean waist circumference (cm) and sum of skinfold thickness according to quintiles of time spent in vigorous intensity activities for boys and girls separately. A significant inverse linear trend was found for boys but not girls. In the whole-sample (boys and girls combined), the mean waist circumference (cm) was 69.9, 70.0, 67.7, 68.6, and 66.8 in quintiles 1 to 5 of VPA, respectively ($p=0.005$) and the mean of sum of skinfolds (mm) was 20 29.6, 28.5, 23.9, 23.6, and 21.4 in quintiles 1 to 5 of VPA, respectively ($p<0.001$) (data not shown in the Figure).

DISCUSSION

In a cross-sectional analysis of data collected in adolescent members of the 1993 Pelotas 25 (Brazil) birth cohort study, we showed that objectively measured physical activity of vigorous

intensity is associated with waist circumference and sum of skinfold thicknesses, while weaker and non-significant associations were found in terms of moderate intensity activities and total body movement defined as accelerometer cpm. Furthermore, associations were stronger in boys than in girls, although there was no statistically significant interaction with
5 sex. These findings suggest that promotion of improvements in body composition in adolescents should focus on vigorous intensity, instead of moderate intensity physical activities.

Our data are in accordance with findings from previous studies in developed societies. In a
10 cross-sectional study, Dencker et al (32) reported that only vigorous intensity activities was associated with body fat determined by X-ray absorptiometry in 8 to 11 years-old Swedish boys and girls. Ekelund et al (13) found a small but significant inverse association between time spent at moderate and vigorous intensity activities and sum of five skinfolds thicknesses, even after adjustment for sex, sexual maturation, birth weight, parental BMI and study
15 location in 9 to 10 year-old Europeans. However, the association between physical activity and BMI was not significant. Ness et al.(14) studied 5500 adolescents aged 12 years. Physical activity was measured by the Actigraph accelerometer and body composition by the Lunar Prodigy dual X-ray absorptiometry scanner. The authors found an inverse association between time spent at moderate to vigorous intensity activities with fat mass, and this association was
20 stronger for boys than girls. In the present study, no significant association among girls was observed. However, this finding must be interpreted with caution as it might be due to lack of statistical power rather than lack of a physical activity effect on body composition. Supporting this rationale is the fact that the direction of the regression coefficients (Table 3) was similar in boys and girls yet the coefficients were weaker for girls. The effects of physical activity on
25 body composition may also be age-dependent, but studies usually include a narrow range of

ages and, therefore, extrapolation of findings to the whole adolescence period is hampered. On the other hand, a narrow age range may aid in discerning the association between physical activity and body composition more reliably, given natural changes in activity patterns during maturation (33, 34).

5

These findings do not necessarily imply that only vigorous intensity physical activities should be promoted among adolescents. A recent review demonstrated several mechanisms by which moderate and vigorous intensity adolescent physical activity can affect health, both in the short and in the long-term (12). However, strategies in which body composition 10 improvements are a priority should take into account the fact that vigorous intensity activities are more likely to be effective. Our data suggest that even modest increases in daily time spent in VPA may have important impact on obesity levels and this is consistent with other studies (14, 32).

15 Some methodological aspects of our study should be considered when interpreting our findings. First, although nested within a prospective cohort study, our analyses are cross-sectional and we cannot infer causality between the associations. Although it is known that physical activity affect body composition, it is also possible that the nutritional status of a given adolescent will either increase or decrease his/her activity levels. Second, we decided 20 not to analyze BMI as an outcome variable, despite it being the most frequent outcome in the literature (8). This decision was based on the fact that our dataset includes variables which are more sensitive than BMI at estimating body composition. Further, it was previously shown that BMI is a poor index of fatness in individuals (9), particularly when exploring the association with physical activity because physical activity practice is associated with 25 decreased fat mass and also with increased fat-free mass. A major strength of our study was to

measure physical activity by accelerometer in a population-based sample from a middle-income country over several consecutive days, including the weekend. Furthermore, we used 5s epoch so that very short bouts of physical activities, which may be typical of adolescents' physical activity behavior could be traced. However, accelerometer measurements have also 5 limitations. Individuals might modify their behavior when using a device (i.e. reactivity). In order to address this issue we compared the self-reported physical activity level of the current study with the 2004 follow up and found similar results. Thus, we believe that our associations are not affected by this issue. Furthermore, motion sensors do not accurately measure some activities (i.e. cycling, upper body movement, and uphill walking) and were 10 not worn during swimming. However, these activities are performed by less than 10% of Brazilian adolescents and thus we are confident in the ability of accelerometer data to rank individuals accurately. Finally, a series of body composition indices were available and rigorous standardization sessions were carried out in order to guarantee data quality.

15 In summary our data showed an inverse association between vigorous intensity activity and waist circumference and sum of skinfolds thicknesses even after adjustment for several potential confounders. Total activity and time spent in MPA were not associated with any of the outcomes in the adjusted analyses. The 1993 Pelotas (Brazil) Cohort is an ongoing study that constitutes a distinctive opportunity to investigate several research questions in a middle-
20 income setting. In future follow-up visits of the same cohort, we plan to collect the same physical activity data and other anthropometric variables. This will allow us to explore the longitudinal association between physical activity measured by accelerometers in early and mid-adolescence and body composition indices to be collected in late adolescence and early adulthood.

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Table 1 – Socio-demographic characteristics, sexual maturity indicators and dietary habits of the sample. The 1993 Pelotas (Brazil) Birth Cohort Study.

	Whole sample n (%)	Boys n (%)	Girls n (%)
Sex			
Boys	265 (51.9)	-	-
Girls	246 (48.1)	-	-
Age (years)			
12	77 (15.1)	39 (14.7)	38 (15.5)
13	431 (84.3)	224 (84.5)	207 (84.1)
14	3 (0.6)	2 (0.8)	1 (0.4)
Socioeconomic level			
A (highest)	16 (3.2)	9 (3.4)	7 (2.9)
B	93 (18.3)	55 (20.8)	38 (15.6)
C	177 (34.9)	88 (33.3)	89 (36.6)
D	187 (36.9)	97 (36.7)	90 (37.0)
E	34 (6.7)	15 (5.7)	19 (7.8)
Tanner maturation stage for amount of hair in genitals			
II	57 (11.3)	31 (12.0)	26 (10.6)
III	118 (23.4)	105 (40.7)	13 (5.3)
IV	271 (53.8)	116 (45.0)	155 (63.0)
V	58 (11.5)	6 (2.3)	52 (21.1)
Tanner maturation stage for size and shape of genitals			
I	15 (3.1)	13 (5.3)	2 (0.8)
II	43 (8.8)	27 (11.0)	16 (6.6)
III	126 (25.8)	65 (26.5)	61 (25.0)
IV	224 (45.8)	92 (37.6)	132 (54.1)
V	81 (16.6)	48 (19.6)	33 (13.5)
Menarche			
Yes	-	-	189 (76.8)
No	-	-	57 (23.2)
Low consumption of fibers in diet			
Yes	70 (13.7)	35 (13.2)	35 (14.2)
No	441 (86.7)	230 (86.8)	211 (85.8)
High consumption of fat in diet			
Yes	260 (50.9)	134 (50.6)	126 (51.2)
No	251 (49.1)	131 (49.4)	120 (48.8)

Table 2. Descriptive analyses of physical activity and body composition data.

	Whole sample (n=457)	Boys (n=238)	Girls (219)	p-value*
Physical activity related-variables				
Mean days wearing the accelerometer	4.1	4.2	4.0	0.009
Mean time spent at sedentary activities (min.day ⁻¹)	660	660	661	0.97
Mean time spent at moderate intensity activities (min.day ⁻¹)	63	69	58	<0.001
Mean time spent at vigorous intensity activities (min.day ⁻¹)	9	11	7	<0.001
Total counts	396511	436924	352592	<0.001
Counts.min ⁻¹	428	463	391	<0.001
Percentage of individuals who practiced $\geq 60\text{min}.\text{day}^{-1}$ of moderate to vigorous intensity activities	61.3%	69.8%	52.1%	<0.001
Body composition related-variables				
Mean triceps skinfold (mm)	14.9	13.1	16.9	<0.001
Mean subscapular skinfold (mm)	10.6	9.3	12.0	<0.001
Mean waist circumference (cm)	68.6	69.5	67.7	0.02
Waist-to-hip ratio	0.79	0.81	0.76	<0.001
Waist-to-thigh ratio	1.46	1.51	1.41	<0.001

* T tests for comparison between sexes.

Table 3. Crude and adjusted regression coefficients (standard error) of the association between physical activity and body composition indices (*whole sample*).

	Accelerometer counts/min	p value	Moderate activities (min/day)	p value	Vigorous Activities (min/day)	p value
Circumference (cm)						
Waist crude	-0.002 (0.003)	0.42	-0.001 (0.01)	0.94	-0.15 (0.05)	0.007
Waist model 1	-0.0008 (0.003)	0.76	0.009 (0.01)	0.53	-0.14 (0.05)	0.009
Waist model 2	-0.003 (0.003)	0.18	-0.007 (0.01)	0.62	-0.20 (0.06)	<0.001
Waist model 3	0.009 (0.003)	0.75	0.02 (0.01)	0.18	-0.15 (0.06)	0.007
Ratios						
Waist-to-hip crude	0.00007 (0.00002)	<0.001	0.0004 (0.0001)	<0.001	0.001 (0.0004)	<0.001
Waist-to-hip model 1	0.00007 (0.00002)	<0.001	0.0004 (0.0001)	<0.001	0.001 (0.0004)	0.001
Waist-to-hip model 2	0.00004 (0.00002)	0.03	0.0002 (0.00009)	0.006	0.0002 (0.0003)	0.56
Waist-to-hip model 3	0.00001 (0.00002)	0.39	0.0001 (0.00009)	0.11	-0.0001 (0.0004)	0.76
Waist-to-thigh crude	0.0001 (0.00004)	0.008	0.0005 (0.0002)	0.02	0.002 (0.0008)	0.01
Waist-to-thigh model 1	0.00009 (0.00004)	0.02	0.0004 (0.0002)	0.05	0.002 (0.0008)	0.02
Waist-to-thigh model 2	0.00002 (0.00003)	0.49	0.0001 (0.0002)	0.46	-0.0002 (0.0007)	0.82
Waist-to-thigh model 3	-0.00002 (0.00004)	0.53	-0.00007 (0.0002)	0.72	-0.0006 (0.0008)	0.38

	Accelerometer counts/min	p value	Moderate activities (min/day)	p value	Vigorous Activities (min/day)	p value
Skinfold (mm)						
TR + SB crude	-0.007 (0.004)	0.07	-0.03 (0.02)	0.28	-0.39 (0.08)	<0.001
TR + SB model 1	-0.005 (0.004)	0.26	-0.008 (0.02)	0.75	-0.38 (0.08)	<0.001
TR + SB model 2	-0.002 (0.004)	0.58	-0.0006 (0.02)	0.98	-0.28 (0.09)	0.002
TR + SB model 3	0.004 (0.004)	0.36	0.04 (0.02)	0.11	-0.20 (0.09)	0.02

TR + SB = sum of triceps (TR) and subscapular (SB) skinfolds.

Model 1 is adjusted for socioeconomic status.

Model 2 is adjusted for sex.

Model 3 is adjusted for sex, socioeconomic status, age, Tanner stage of sexual maturation, diet pattern, height, and birth weight.

Table 4. Crude and adjusted* regression coefficients (standard error) of the association between physical activity and body composition indices in boys and girls.

	BOYS						GIRLS					
	Accelerometer counts/min	p	Moderate activities (min/day)	P	Vigorous Activities (min/day)	p	Accelerometer counts/min	p	Moderate activities (min/day)	p	Vigorous Activities (min/day)	
Circumferences (cm)												
Waist crude	-0.007 (0.003)	0.05	-0.03 (0.02)	0.11	-0.21 (0.07)	0.002	0.001 (0.004)	0.76	0.02 (0.02)	0.28	-0.18 (0.10)	0.09
Waist adjusted	0.002 (0.003)	0.51	0.02 (0.02)	0.34	-0.09 (0.06)	0.17	0.003 (0.004)	0.50	0.02 (0.02)	0.28	-0.15 (0.10)	0.15
Ratios												
Waist-to-hip crude	0.00004 (0.00002)	0.03	0.0003 (0.0001)	0.007	0.0004 (0.0003)	0.23	0.00003 (0.00003)	0.26	0.0002 (0.0002)	0.18	-0.0003 (0.0007)	0.67
Waist-to-hip adjusted	0.00002 (0.00002)	0.23	0.0002 (0.0001)	0.06	0.0002 (0.0004)	0.53	0.00001 (0.00003)	0.61	0.0001 (0.0002)	0.53	-0.0003 (0.0007)	0.61
Waist-to-thigh crude	-0.000004 (0.00004)	0.93	0.00007 (0.0002)	0.78	-0.0003 (0.0008)	0.74	0.00007 (0.00006)	0.25	0.0002 (0.0003)	0.43	0.00009 (0.001)	0.95
Waist-to-thigh adjusted	-0.000006 (0.00005)	0.22	-0.0002 (0.0003)	0.53	-0.0009 (0.0009)	0.37	0.00003 (0.00006)	0.66	0.00006 (0.0003)	0.84	-0.0004 (0.001)	0.75

	BOYS						GIRLS					
	Accelerometer counts/min	p	Moderate activities (min/day)	P	Vigorous Activities (min/day)	p	Accelerometer counts/min	p	Moderate activities (min/day)	p	Vigorous Activities (min/day)	
TR + SB crude	-0.006 (0.005)	0.27	-0.03 (0.03)	0.30	-0.30 (0.11)	0.005	-0.006 (0.005)	0.27	0.04 (0.03)	0.23	-0.22 (0.16)	0.17
TR + SB adjusted	0.003 (0.006)	0.59	0.02 (0.03)	0.60	-0.17 (0.11)	0.14	0.009 (0.006)	0.15	0.06 (0.03)	0.09	-0.12 (0.15)	0.43

TR + SB = sum of triceps (TR) and subscapular (SB) skinfolds

Adjusted for socioeconomic status, age, Tanner stage of sexual maturation, diet pattern, height, and birth weight.

Titles and legends to Figures

Figure 1. Mean of waist circumference (cm) and sum of skinfold thickness according to quintiles of time spent in vigorous-intensity physical activities (VPA) in boys and girls.

The mean time (min/day) of VPA in the whole sample was 2.1, 4.7, 7.4, 11.1, and 20.9 to quintiles 1 to 5, respectively. Black bars represent boys while white bars represent girls.

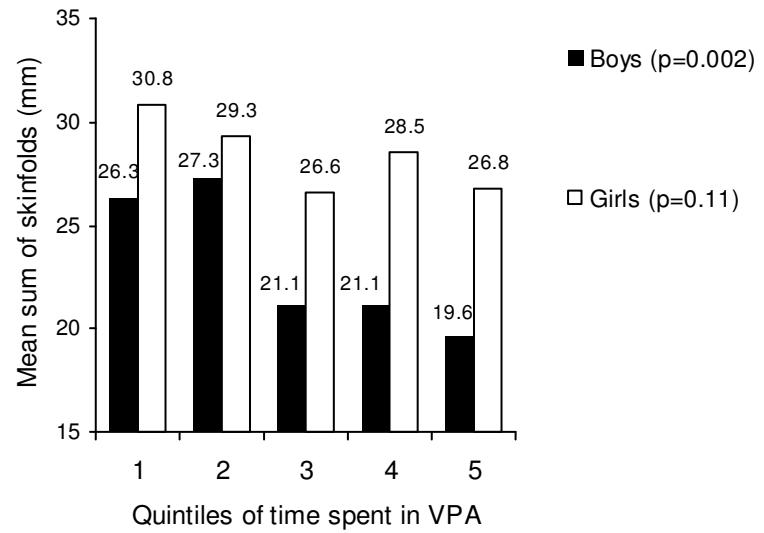
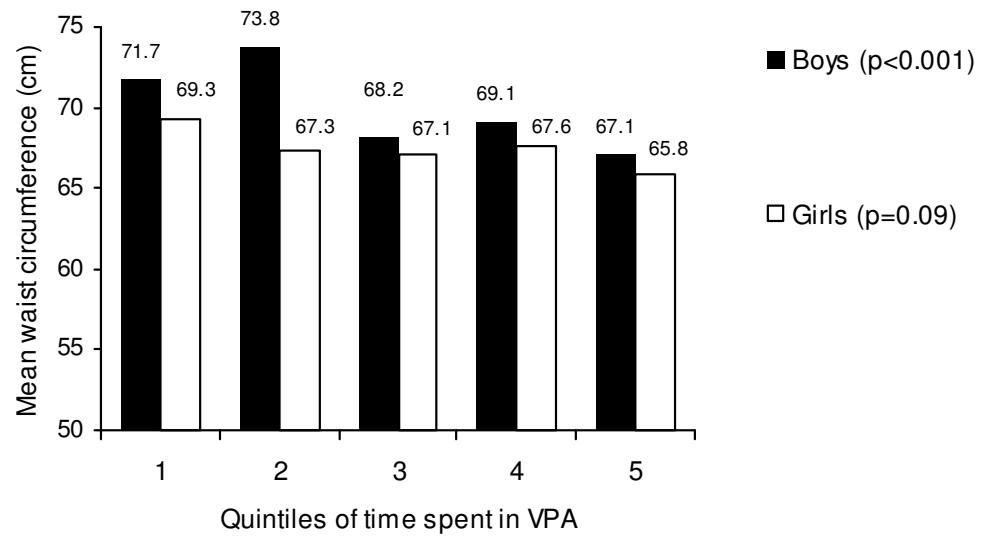


Figure 1

**Physical activity as a predictor of adolescent body fatness: a systematic
review**

Aceito na Sports Medicine

19 May 2008

RE: SPO-S-07-01422R1, entitled "Physical activity as a predictor of adolescent body fatness: a systematic review"

Dear Mr. Reichert,

Further to our last e-mail regarding your revised manuscript, we have now assessed the manuscript and it has been accepted and scheduled for publication in Sports Medicine, subject to your consideration of any points or queries we may need to bring to your attention during our inhouse copy and technical edit. We will get back to you with our own editorial comments in due course.

Your article is currently scheduled for issue 39 (4) of Sports Medicine, which should be dispatched in March 2009. You will receive a copy of the proofs for approval prior to publication. Please accept our sincere apologies for the delay in publishing your manuscript. There is currently a huge amount of interest in the journal and we are therefore having to deal with a large number of articles at the pre-publication stage. We need to prioritise publication based on both timeliness and article mix to maximise reader interest in each issue of the journal. We realise that this situation must be very frustrating for individual authors and for this we apologise.

When we do send you the editorial comments or proofs, we would welcome the addition of any important new findings that may have been published in the literature during this interim period. However, we often request a very short turnaround time for your reply to ensure that we meet our editorial deadlines. Consequently, I would ask you to continue to monitor the literature related to the topic. In this way, the incorporation of any new data may be made expeditiously when we contact you again.

Please could you confirm whether table II has been previously published in reference 13 (Connelly JB, Duaso MJ, Butler G. A systematic review of controlled trials of interventions to prevent childhood obesity and overweight: a realistic synthesis of the evidence. Public health. 2007 Jul;121(7):510-7)? If so, we will require permission from the publisher to reproduce it.

Meanwhile, thank you for the obvious time and effort you have put into the preparation and revision of this article.

Please feel free to contact me with any questions.

With kind regards

Emma Pearl

Publication Manager, Sports Medicine

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Physical activity as a predictor of adolescent body fatness: a systematic review

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ABSTRACT

Adolescent obesity has increased dramatically in several countries in recent decades, however the contribution of physical activity level to adolescent adiposity requires clarification. The aim of this review was to investigate the effect of physical activity on subsequent levels of adiposity in adolescence. We also evaluate methodological aspects of the studies included in the review, particularly in terms of measurement accuracy for both exposure (physical activity) and outcome (adiposity) variables. Systematic searches of the literature were undertaken using online databases, including Pubmed/Medline, examination of citations, and contacting of authors. The online databases were searched from their earliest records until 2007. Only longitudinal studies with 50 or more adolescents were included. Two independent reviewers assessed the quality of the studies using the Downs & Black checklist. Thirteen observational, five experimental and six quasi-experimental studies (without control group) were identified. Almost all studies were carried out in high-income settings and showed protective effects of physical activity for both prevention and treatment of adolescent obesity. However, experimental studies undertaken with obese adolescents at baseline usually combined physical activity with dietary changes, making difficult to assess the effect of physical activity itself on the treatment of obesity. Physical activity estimated from questionnaires and body mass index (BMI) were the most frequently measures. Despite the feasibility of using these approaches in epidemiological studies, significant limitations are evident. Questionnaires are subjective and adolescents may not report physical activity level accurately. Furthermore, BMI is not an accurate measure of fatness for adolescents, as it is also associated with lean mass, hence bias may arise from its longitudinal association with physical activity level. We conclude that despite the majority of studies reviewed showing protective effects of physical activity on adiposity, particularly in individuals obese at baseline, the current literature on this issue is sparse and several methodological drawbacks are evident. The main limitations relate to a lack of validity in the measurements of both physical activity and body composition. Further studies are needed in order to generate evidence-based recommendations for the quantity and quality dose of adolescent physical activity required to prevent or treat adolescent obesity.

Keywords: motor activity; adiposity; obesity; youth; exercise; body composition; longitudinal studies

1. INTRODUCTION

Adolescent obesity has increased dramatically in several countries in recent decades (1). Currently, high rates of excessive body weight are observed not only in developed societies but also in low- and middle-income countries. For example, in Brazil the prevalence of overweight more than tripled in boys aged 10 to 19 years (from 2.6% to 11.8%) and more than doubled in girls (from 5.8% to 15.3%) from 1975 to 1997 (2).

Obesity in adolescence is associated with a broad range of adverse health effects. First, adolescent obesity has been shown to track strongly into adulthood , and the health consequences of adulthood obesity are well established (3). More recently, studies have demonstrated that some diseases, including elevated blood pressure, type 2 diabetes, asthma, and sleep disorders are more frequent among obese than non-obese adolescents (4-7) . Obesity is also associated with psychological disorders (8) .Thus, studies investigating the factors that play a role in the prevention or treatment of adolescent obesity are warranted.

The rapid change in the prevalence of obesity worldwide suggests that factors other than genes play an important role in the aetiology of obesity, although an interaction between these factors may occur. Fat accumulation is ultimately the result of chronic positive energy balance (energy intake > energy expenditure). In this context, physical activity, which accounts for a large part of total energy expenditure, is predicted to influence adiposity. Several cross-sectional studies have addressed this issue; however, their results are conflicting (9-12). Such lack of consistency may arise because the association between physical activity and adiposity is highly susceptible to reverse causality (adolescents may change their physical activity level depending on the degree of adiposity). Theoretically, longitudinal studies might also be affected by reverse causality but this bias is much less likely in randomized controlled trials. Thus, longitudinal studies are more appropriate to investigate this issue. Nonetheless, longitudinal studies performed during the period of adolescence (10 to 19 years) are rare and very heterogeneous concerning many aspects which may likewise lead to conflicting findings.

Few systematic reviews on the association between physical activity and adiposity in youth have been carried out and overall, their results indicate that increased physical activity may play a role in both prevention and treatment of obesity. Not only did some reviews include only experimental studies (13, 14) and one included only observational studies (15), none of them focused exclusively on adolescents.

Physical activity can be subdivided into sleep, sedentary behaviours and motion behaviours. We carried out a systematic review of the literature with the primary aim of investigating the effect of physical activity, specifically the motion aspect, on subsequent levels of adiposity in adolescents. Our review included both observational and experimental studies. We also evaluated methodological aspects of the studies included in the review, particularly in terms of measurement accuracy of both exposure (physical activity) and outcome (adiposity) variables. This issue was not addressed in any previous review, but is critical for interpreting the results of a collation of studies using heterogeneous methods.

2. METHODS

In July 2007, the following electronic databases were searched from the earliest record: MEDLINE, SPORTDiscus, SCIELO, BioMed Central and PsycInfo. The reference lists from identified articles were searched manually. The first author of the articles included was contacted and questioned about other published or unpublished data. The following keywords were used: “abdominal fat”, “abdominal obesity”, “adiposity”, “body composition”, “body fat”, “body fat distribution”, “body mass index”, “central adiposity”, “central fat”, “central fatness”, “central obesity”, “centrally-distributed fat”, “centrally-distributed obesity”, “fat”, “fat patterning”, “fatness”, “metabolic syndrome”, “metabolic syndrome x”, “obese”, “obesity”, “syndrome x”, “truncal fat”, “truncal obesity”, “trunk adiposity”, “trunk fat”, and “trunk obesity”. These keywords were combined with “exercise”, “inactivity”, “motor activity”, “physical activity”, “physical exercise”, or “sports”.

Given that the aim of the present review was to assess the role of physical activity during adolescence on subsequent levels of adiposity, cross-sectional studies were excluded because of their inability to establish temporality. The following keywords were thus used: “clinical trial”, “cohort”, “experimental”, “experimental design”, “follow-up”, “intervention”, “intervention studies”, “longitudinal”, “panel”, “prospective”, and “trial”. The keywords “adolescence”, “adolescents”, “teenager” and “youth” were also used in the literature search. Studies were considered if the outcome variable (adiposity) was collected when subjects were aged 10 to 19 years, even if the exposure (physical activity) had been measured before 10 years of age. This strategy was adopted since too few studies were carried out exclusively during adolescence. However, studies were only considered if most of the ages of the participants were within the adolescence period. Studies with fewer than 50 subjects in the sample were not considered because of the low statistical power associated with small samples.

The search resulted initially in 571 papers. After reading the titles and abstracts, 43 papers were selected, and after reading the full texts, 19 papers were included in the review. A further five papers were selected through the reference lists of these articles, and contact with experts in the area.

Two independent reviewers assessed the quality of the studies using the Downs & Black checklist (16). In case of eventual differences between the two reviewers, the papers were re-assessed until both referees agreed with the evaluation. Because the original Downs & Black checklist is applicable to experimental designs, a modified version of the scale (17) was used to assess the quality of observational studies (table II). Therefore questions 8, 13, 23, and 24 of this instrument were not considered for observational studies. All questions were coded as zero (representing poor quality) or one, with the exception of question five, which was coded with zero, one, or two. Furthermore, question 27, originally coded from zero to five was dichotomized into zero or one (code one was attributed to studies which mentioned a statistical power equal or greater than 80%). The

final scale ranged from zero (poorest quality) to 24 (best quality) points for observational studies or 28 points for experimental studies.

3. RESULTS

Table I presents a description of the articles included in the review, summarizing the studies included. Studies are presented according to the design and in alphabetical order of the first author's name. A total of 5 experimental (18-22), 6 quasi-experimental (without control group) (23-28), and 13 observational (cohorts) studies (29-41) were found and fulfilled the inclusion criteria. Only five studies exclusively included adolescents (individuals aged 10 to 19 years) and all but one (35) study were undertaken in high-income countries. Sample sizes ranged from 55 (23) to 11887 (30), and follow-up duration was as short as 3 months (18) and as long as 9 years(34). Table 2 describes the methodological quality of the papers according to the Downs & Black checklist. Figure 1 presents potential confounders considered by the studies reviewed.

3.1 Measuring the exposure: physical activity

In the 24 studies revised here, physical activity was both measured, and also promoted in the intervention studies, in a variety of different ways.

Experimental and quasi-experimental studies

All experimental and quasi-experimental studies included components other than a physical activity program in the intervention (table I). For example, Gutin et al (19) assigned participants to one of three groups: a) 1h lifestyle education every two weeks (which served as a control condition because it was offered to all groups); b) lifestyle intervention plus moderate physical activities (55-60% of peak oxygen uptake - $VO_{2\text{peak}}$); and c) lifestyle intervention plus high-intensity physical activities (75-80% $VO_{2\text{peak}}$). Energy expenditure for each session was held at approximately 250kcal/session, therefore, the training duration was related to physical activity intensity. However,

individuals of the high intensity group performed the exercises in a heart rate (154beats/min) significantly lower than that prescribed (167beats/min) and the attendance rate was low for both physical training groups (~54%).

Savoye et al. (21) randomly assigned overweight individuals to either a control group (receiving traditional clinical weight management counseling) or a 12-month weight management group, which received a family-based program involving exercise, nutrition, and behavior modification. The exercises consisted of aerobic activities performed at 65-85% of the age-adjusted maximal heart rate for twice a week (50min each session) during the first six months and 100min per month for the last six months.

The intervention proposed by Reinehr et al (25) was based on one year of physical exercise, nutrition education and behavior therapy for children and parents separately (first three months) and individual psychological care of the child and its family. Twenty out of the 75 participants dropped out during the study, but training session attendance among those who remained in the study was > 90%.

Sothern et al (26) also proposed an intervention with three main branches: nutrition, physical exercises, and behavior modification. The exercise was of moderate intensity (45-55% of $\text{VO}_{2\text{max}}$) and the frequency and duration of the sessions varied according to the degree of obesity, although no specific information for these variables was provided. Ninety three percent of individuals completed the acute phase (10-20 weeks) of intervention and 62.5% completed the 1-year program. Mean attendance during the acute phase was 91% with 57% for the remaining phase.

Observational studies

All observational studies estimated physical activity level by questionnaires (Table I). Most of the questionnaires were self-reported, with the remainder completed by parents (33, 35) or teachers (41).

3.2 Measuring the outcome: adiposity

Adiposity was also measured in a variety of different ways in the 24 different studies.

Experimental and quasi-experimental studies

Most of the studies included more than one method to estimate adiposity. Body mass index or change in this variable during the follow-up period was the most frequently used outcome (18, 21, 24, 25, 28). One study defined obesity based on both BMI and tricipital skinfold thickness (22). Percentage of body fat derived by either skinfold thickness (26, 27) or bioelectrical impedance (21) was another outcome frequently used in the studies. Dual-energy X-ray absorptiometry (DEXA) was used in two studies (19, 23) and magnetic resonance in one study (19).

Observational studies

Observational and experimental/quasi-experimental studies used similar methods to estimate adiposity. Virtually all observational studies used BMI as a measure of adiposity (Table I), with 6 of the 13 studies using only this method. Skinfold thickness measurements and bioelectrical impedance, as well as circumferences were also used in some studies. One study (36) used DEXA.

3.3 Effects of physical activity on adiposity measures

Experimental and quasi-experimental studies

All studies showed favorable effects of the intervention on adiposity level of adolescents. However, it should be highlighted that all interventions included other exposures besides physical activity (eg dietary change). Thus, it is difficult to assess the independent impact of physical activity on adiposity. One school-based intervention decreased obesity only

among girls (22), while the remaining studies found comparable results of the intervention among both sexes.

One experimental study measured total body composition by DEXA and visceral adipose tissue and subcutaneous abdominal tissue by magnetic resonance imaging (19). Based on efficacy analyses adjusted for potential confounders and baseline values, the authors showed a significant decline in the following outcomes after 8 months of physical training: visceral adipose tissue ($-42.0 \pm 9.3\text{cm}^3$), subcutaneous abdominal adipose tissue ($-69.7 \pm 55.9\text{cm}^3$), and percentage of body fat ($-3.57 \pm 0.80\%$). The efficacy analyses included only those participants who attended at least 40% of intervention sessions. However, the intention-to-treat analyses (i.e. effectiveness analysis) showed no association of the intervention with either of the outcomes. Furthermore, the authors highlighted that there was no evidence that the high-intensity physical training was more efficacious than the moderate-training, although no analyses for this association were shown.

Observational studies

All but two studies (39, 40) showed significant inverse associations of physical activity with body composition or BMI, although the magnitude of the association was markedly different between studies. Some studies suggest that the effect of physical activity on these outcomes depended on gender (29, 36), ethnicity (34) and baseline BMI (30). For example, Berkey et al (29) showed that active girls had smaller gains in BMI ($-0.0284; p = 0.046$) over a one-year period. Among boys this difference was not significant, despite similarity in the strength and direction of the effect (coefficient= $-0.0261; p=0.094$). In contrast, Mundt et al. (36) showed that physical activity was associated with reduced increments in fat mass (measured by DEXA) in boys but not girls.

3.4 Prevention versus treatment effects

Experimental and quasi-experimental studies

The evidence that physical activity prevents adolescence obesity is very limited, since only two experimental studies on normal weight individuals were located (20, 22) and one of them found no effect of the intervention on boys (22). The remaining experimental and quasi-experimental studies were carried out with either overweight or obese individuals at baseline and showed positive effects of the intervention (18, 19, 21, 23-28). Overall, the results were consistent regardless the method used to estimate adiposity. However, McMurray et al (20) found no differences in the BMI changes among four groups studied (educational intervention, exercise intervention, educational plus exercise interventions and control group). In contrast, the sum of skinfolds increased less in the exercise intervention groups than in the control and education only groups ($p<0.001$).

It is very difficult to draw confident conclusions regarding the actual effects of physical activity from these studies because the interventions also focused on other aspects, for example diet. Therefore, although there is some evidence that physical activity is important in the prevention and treatment of adolescence obesity, the real impact of physical activity, as well as the type, frequency, and duration that is most beneficial remains unknown.

Observational studies

Observational studies demonstrated that physical activity might play a role in the prevention of fat accumulation in normal-weight subjects. For example, Kimm et al. (34) demonstrated a clear dose-response effect of physical activity practice on skinfold thicknesses: girls who were more active from ages 9 to 19 years had smaller gains in skinfold thicknesses throughout adolescence. However, although most studies estimated physical activity from questionnaires, there are numerous differences between these instruments. Some of them estimated only leisure-time physical activities, while others

measured all-domain activities. They also used a variety of cut-off points and units of measures (metabolic equivalents, minutes of physical activity and kilocalories spent per week, etc). Another pitfall of questionnaires concerns their subjectivity. Therefore, the relative importance of type, frequency, and duration of physical activities for preventing or treating obesity is also unknown.

3.5 Measuring potential confounders

All observational studies adjusted their analyses for potential confounders. Figure 1 shows the factors most frequently considered as confounders and the number of studies that included them in analyses. The figure does not show gender as a potential confounder, but most of the studies performed analyses stratified by this variable. Confounders not included in the figure, but considered by at least one study, were smoking habits (34), hours of television viewing, number of parents (31), compensatory behaviors (i.e. vomiting for weight-control purposes, laxative abuse, and diuretic abuse) and depressive symptoms (40) and school (38).

Table II describes the methodological quality of the articles, as defined by the Downs and Black modified scale. The average quality score of the observational studies was 16.4/24 ($sd = 2.1$; median 17; range 12 to 19) and of the experimental studies was 17.2/28 ($sd = 2.7$; median = 18.0; range 14 to 21).

DISCUSSION

This review focused on the role of physical activity (or lack of activity) on subsequent levels of adiposity with the latter, at least, being investigated during adolescence. Despite most of the papers reviewed showing protective effects of physical activity against adiposity, several limitations are evident in the literature. Although the longitudinal relationship between these variables is of most importance, few studies on this subject (particularly experimental studies) could be located. Furthermore, there are virtually no relevant studies from low- or middle-income countries. This result was expected, given

the fact that longitudinal studies are very expensive and time consuming. It is plausible that the physiological effects of a specific physical activity dose will have similar effects on body composition regardless of the population and setting. However, different activities are practiced in low- and middle-income country populations compared to those of high-income countries (e.g. leisure-time versus travel or subsistence activity) (42-45). Therefore it is important to assess the effectiveness of different activity patterns on changes in body composition. The only study undertaken outside high-income countries from Thailand (35) showed similar results (i.e. an increase in BMI was associated with lower levels of exercise), however many populations and settings still remain unexplored.

The best study design for testing the hypothesis that physical activity prevents excessive fat accumulation is randomized field trials. However, virtually all available data regarding the prevention of fat accumulation through increased physical activity derive from observational studies, and several biases are therefore of concern. For example, high rates of loss of follow up and refusal to participate are observed in the reviewed literature, which may lead to an overestimation of the actual effects of physical activity. All but two (20, 22) of the experimental (randomized intervention) and quasi-experimental studies reviewed were carried out in individuals overweight/obese at baseline. Although all of these studies suggested favorable effects of the intervention on adiposity levels, such studies are very heterogeneous regarding both exposure and outcome and their results must be interpreted with caution. First, none of the studies verified the effect of physical activity itself on adiposity levels. Interventions usually consisted of a combination of factors such as change in dietary habits, behavior, and physical activity levels (18, 21, 23, 25, 27, 28). Thus, the results are likely to be a consequence of an interaction between these variables. Second, there were noticeable differences in terms of baseline body composition, duration of the intervention, and number of subjects included in the study, which all may affect the results.

An important methodological aspect to be considered is the decision of adjusting or not for the baseline value of the outcome. One should consider that physical activity practice may have different consequences on later body composition depending on the current

nutritional status of the individual. In order to address this issue, one of the possibilities is to adjust the association between physical activity and later body composition by baseline values of the outcome variable. An alternative approach is to test interactions between the variables. Both approaches were rarely used in the reviewed studies.

Regardless of the design of the study, a further important limitation relates to the measurement of both physical activity and body composition. Despite the recognized importance of these variables for patterns of morbidity and mortality worldwide, accurate determination of physical activity and body composition in large-scale studies remains a challenge. A non-systematic error in the measurement of any of these variables would underestimate the effect of physical activity; however, the effect of a systematic error is unknown.

The optimum technique for assessing fat mass is the multi-component model, although deuterium dilution is also considered to have high accuracy (46). Magnetic resonance imaging (MRI) is also considered a gold standard for adipose tissue quantity and distribution, though it should be noted that adipose tissue is not equal to fat mass, hence reducing comparability across studies. DEXA is often described as an accurate objective technique, however bias is systematically associated with factors such as gender, body size and obesity status (47, 48).

In this context, we found that BMI was the most frequently outcome (18 out of 24 studies reviewed). It has been shown that for a given value of BMI, a wide range of fatness is observed in children (49). The limitation of BMI is much more evident if the aim is to investigate its association with physical activity. Physical activity can increase lean mass as well as decrease fat (50). However, BMI does not distinguish between these two compartments, and, therefore, either stability or even increase in this index may actually correspond to favorable changes in body composition. In fact, the lack of association between physical activity and BMI in boys observed in some studies (29, 33) may be related to this issue. Nonetheless, one should note that studies that used BMI and another method to estimate adiposity (i.e. bioelectrical impedance, skinfold thickness or

circumferences) usually found similar effects of physical activity regardless the outcome measurement. For example, Savoye et al (21) showed an effect of comparable magnitude ($p<0.001$) of physical activity on BMI and percentage of body fat derived from a body fat analyzer (Tanita, TBF 300).

Skinfold thickness was another method frequently used as outcome. This approach at least measures one component of adiposity directly, but may not reflect deeper fat depots (51) which are most strongly associated with health outcomes (52). Further, published equations have been shown to be inaccurate. However, the majority of adolescent fat mass is subcutaneous rather than intra-abdominal, hence raw skinfold data provide valuable information about energy stores. Raw data expressed as standard deviations are frequently based on reference data that may not be appropriate for contemporary populations (53, 54), however ranking within the population is likely to be accurate.

Some studies, with noticeably lower number of participants, have used more sophisticated methods such as DEXA. In addition to the limitations in accuracy discussed above, their results are frequently converted into percentage of body fat. However, percentage of body fat has been criticized because it is both statistically and conceptually problematic (54-56). For example, %fat is the inverse of %lean mass, hence the actual body component associated with other variables remains unclear. Proposed alternatives, such as adjusting body fat to body size (as kg/m^2 , for example) have not been associated with physical activity yet in longitudinal studies, however in cross-sectional studies, both fat mass and lean mass adjusted for height have been associated with physical activity level (57).

Similar lack of validity is also evident in the measurement of physical activity. The gold standards for estimating physical activity in children and adolescent have been claimed to be direct observation, double labeled water, or indirect calorimetry (58). None of the studies used any of these methods. Instead, most studies estimated physical activity by questionnaires. Although questionnaires have some advantages over other methods, they are very subjective. Collecting valid data through questionnaires depend on the reliability

of the interviewee in reporting accurately the practice of physical activities over a determined period of time. This is particularly problematic with children and adolescents, given their low ability to record their activities. Furthermore, physical activities in these ages are generally characterized by irregular bouts of short duration and varied intensity activities, making it even more difficult to obtain accurate data.

An alternative to overcome these shortcomings would be the application of more objective measures of physical activity, such as motion sensors to determine physical activity level. Such techniques have been shown to be valid and reliable in young populations, with no evidence of high reactivity (59). However, our review did not identify any studies using these approaches. Possible reasons for this finding are the high costs associated with the use of such devices in large scale studies, or the limited feasibility of assessing physical activity over long time periods using these instruments. The costs and the storage capacity of recent accelerometer models have changed favorably and it is possible that in the near future these devices will be used in epidemiological studies.

Experimental and quasi-experimental studies usually focused on aerobic activities lasting at least 30min and performed at least twice a week. Gutin et al (19) verified the effect of different intensities on body composition and concluded that either high- or moderate-intensity activities impacted on body composition similarly. However, these authors highlighted important limitations such as the low rate of attendance at the exercise sessions, and the inability to perform the exercises in the target heart rate zone, which might have compromised the results. In this context, based on our review, the current recommendation of 60min/day on most days of week of physical activities practice to prevent/treat adolescent obesity (60) lacks evidence. In fact, this recommendation has been criticized for the same reason in other studies (61).

Some methodological aspects of our review should be highlighted. Several studies on the association between physical activity and body composition were cross-sectional, and thus were not included in this review. Although cross-sectional studies are valuable for

addressing various research questions, they are unable to establish temporality of the association between the exposure and outcome under study (physical activity and adiposity levels), which was the objective of our review. Therefore, our review only included prospective studies, since they are less likely to be affected by reverse causation than cross-sectional studies. Studies with fewer than 50 participants were excluded, due to the lack of statistical power associated with such a low number of individuals. Furthermore, we were interested in the effectiveness (i.e. *intention-to-treat* analysis) rather than efficacy of physical activity, whereas smaller studies usually carry out only efficacy analyses. However, it should be noted that small-sized studies usually allow for more sophisticated measures of both physical activity and adiposity and their results can represent valuable pilot data requiring confirmation in larger samples.

The likelihood of publication bias in our review must be considered. If studies with inconclusive results or indicating an unexpected association (i.e. unfavorable effects of physical activity on adiposity) were not located but do exist, then the beneficial effects of physical activity on adiposity would be overestimated. In order to decrease the likelihood of this bias, several strategies of the literature search were adopted and the authors of the papers included were asked about other studies (either published or unpublished) on the same topic. Finally, we assessed the methodological quality of the studies using a modified version of the Downs Black Scale (16). Such a scale has been used in other reviews (17, 62-64). Overall, results from studies with higher scores were similar to those with lower scores.

Although it is important to have an indicator of the quality of the studies included in the review, some limitations of the Downs & Black Scale itself must be pointed out. First, some criteria (for example #14, #15, and #19) are not applicable to observational studies and were coded as zero (thus, rated as “inadequate”). Second, the absence of some items of the scale in a paper might be a reflection of factors other than solely poorer methodological quality. For example, it is reasonable to believe that authors from small sample-sized studies are under greater editorial pressure to discuss the power of analyses than authors from larger studies. In fact, several large studies were rated as inadequate in

criteria #20 because sample size calculations were not presented. Some items of the scale are vague and difficult to evaluate. For example, regarding criteria #19, there is no widely acceptable rate of desired compliance. Likewise, some studies did not clearly indicate the compliance rate with the intervention. Therefore, we decided to rate all studies as adequate for this item, as this is also recommended in the original article reporting the scale, being relevant whenever misclassification error is likely to bias the association to the null (16).

5. CONCLUSIONS

In summary, this review focused on the longitudinal association between physical activity and adiposity in adolescence. Most studies showed protective effects of physical activity against adiposity, mainly in individuals obese at baseline. Nonetheless, few studies, in particular experimental ones, are available and several methodological drawbacks are evident. The main limitations relate to a lack of validity in the measurement of both physical activity and body composition. Thus, based on the current available data we conclude that the literature offers only limited support for a causal link between physical activity and adiposity in adolescence. Further studies are needed in order to generate evidence-based recommendations for the quantity and quality dose of adolescent physical activity to prevent and treat adolescent obesity.

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Figure captions:

Figure I: Potential confounders considered in the observational studies reviewed.

Table I. Characteristics of the studies included in the review according to design, first author's name, country, sample size, follow-up duration, weight status of the sample, definition of exposure and outcome and main results.

Study (country)	Sample size	Follow-up duration	Weight status of the sample	Definition of the exposure	Definition of the outcome	Main results
EXPERIMENTAL STUDIES						
Gortmaker (1999) (USA)	1295 individuals	21 months	General population	School-based intervention aimed to change PA and dietary habits	Combination of BMI and tricipital skinfold	Intervention was successful among girls but not boys
Eliakim (2002) (Israel)	177 individuals aged 6 to 16 years	3 months	Obese	Dietary-behavior-exercise intervention	BMI	BMI decreased in 74% of the individuals (from $26.1 \pm 0.3 \text{ kg.m}^{-2}$ to $25.4 \pm 0.3 \text{ kg.m}^{-2}$).
Gutin (2002) (USA)	80 adolescents aged 13 to 16 years	8 months	Obese	Lifestyle intervention plus either moderate or vigorous PA	Total (DEXA) and visceral adiposity (magnetic resonance)	High- and moderate-intensity PA were similarly effective in reducing visceral and total-body adiposity
McMurray (2002) (USA)	1140 adolescents (630 females) aged 11 to 14 years	8 weeks	Normal	Aerobic exercise program and education in schools	Sum of skinfold thickness	Exercise group had smaller gains in skinfold thickness than control group
Savoye (2007) (USA)	209 individuals aged 8 to 16 years-old	1 year	Overweight	Weight management family-based program including exercise, nutrition and behavior modification	Change in weight, BMI, and body fat estimated from Tanita, TBF 300	Individuals in the intervention group presented better indicators in all outcomes

QUASI-EXPERIMENTAL STUDIES						
Dao (2004) (France)	55 individuals aged 9 to 17 years	6 to 12 months	Obese	Dietary and physical activity program	Total and regional body composition determined by DEXA	Body fat decreased in both sexes and steepest declines were observed in the trunk.
Reinehr (2003) (Germany)	75 individuals aged 7 to 15 years	1 year	Obese	Intervention consisted of physical exercise, nutritional course and behavior therapy for participants and their parents	BMI	Participation in exercise groups was associated with a decrease in standard deviation scores of BMI
Reinehr (2007) (Germany)	170 individuals (mean age 10.5 years)	1 year	Obese	1 year of physical exercises and 3 months of nutrition education	Standard deviation score in BMI in the 3 rd year after intervention	66% of individuals presented a reduction of standard deviation score in BMI 3 years after the end of intervention
Sothorn (1999) (USA)	87 individuals (39 boys) aged 7 to 17 years	1 year	Obese	Intervention consisting of dietary restrictions, moderate-intensity PA and behavior modification sessions	Percentage of body fat	All individuals presented better body composition indicators

Sothern (2000) (USA)	56 individuals aged 7 to 17 years	1 year	Obese	Dietary restrictions and physical activities	Variation in body weight and percentage of body fat, estimated by skinfold thickness	Individuals had a significant decline in all outcomes after 10 weeks of the program, which was maintained at 1-year follow-up.
Wong (1997) (Singapore)	112 adolescents	2 years	Obese	Weight control programme	BMI	Long-term weight loss was associated with increased PA after intervention
OBSERVATIONAL STUDIES						
Berkey (2000) (USA)	6149 girls and 4620 boys aged 9 to 14 years of age at baseline	1 year	General population	Self-reported PA estimated by questionnaire	1-year change in BMI	PA was inversely associated with large increases in BMI in girls only
Berkey (2003) (USA)	6767 girls and 5120 boys aged 10 to 15 years	1 year	General population	Self-reported PA estimated by questionnaire	1-year change in BMI	PA effects were sex-dependent and stronger in overweight than normal weight adolescents The effect of PA on BMI was stronger than the effect of sedentary activities
Elgar (2005) (UK)	355 adolescents (mean age 12.3 years at baseline)	4 years	General population	Self-reported PA estimated by the Health Behavior of School-aged Children Questionnaire	BMI	Baseline PAL was associated with BMI change but no BMI at follow up

Heelan (2005) (USA)	320 individuals aged 10.2±0.7 years	6 months	General population	Active commuting to and from school estimated from the Self-administered PA Checklist	BMI and body fat determined by skinfold thickness	Significant positive correlation between active commuting and overweight was observed
Kettaneh (2005) (France)	436 individuals aged 8 to 18 years	2 years	General population	Self-reported and parent-assisted PA estimated from the Kriska's modifiable Activity	BMI, sum of skinfold thickness, and waist circumference	A decline in PAL during the follow up period was associated with lower adiposity in girls but not boys
Kimm (2005) (USA)	2379 girls followed up from 9 or 10 to 18 or 19 years old	9 years	General population	Self-reported PA estimated from the Habitual Activity Questionnaires	BMI and sum of skinfold thickness	Active girls had smaller gains in both outcomes than inactive ones
Mo-suwan (2000) (Thailand)	2252 schoolchildren aged 5 to 16 years at baseline	5 years	General population	Exercise level compared with other individuals of the same age, as reported by the parents	BMI	Lower level of exercise was associated with increases in BMI
Mundt (2006) (Canada)	208 individuals aged 8-19 years	Maximum of 7 years; median of 5 years	General population	Self-reported PA estimated from the PA Questionnaire for Children or Adolescents	Fat mass determined by DEXA	PAL was negatively associated with fat mass accumulation in boys, but not girls.
Must (2007) (USA)	173 pre-menarcheal girls, 8 to 12 years old followed up until 4 years post-menarche	7.5 years (average)	General population	Self-reported PA estimated from questionnaire	Percentage of body fat estimated from bioelectrical impedance and	PA was negatively associated with percentage of body fat only among those whose had at least one parent overweight

					BMI	
O'Loughlin (2000) (Canada)	2951 schoolchildren from 9 to 12 years at baseline	1 or 2 years	General population	Self-reported PA estimated from questionnaire	BMI	Highest decile of change in BMI was more frequent among those with low levels of physical activity
Rosenberg (2006) (USA)	1083 students from the fourth grade of seven elementary schools	2 years	General population	Self-reported active commuting estimated from questionnaire	BMI and skinfold thickness	Active commuting to school over 2 years was not associated with BMI change or overweight status.
Stice (2005) (USA)	496 girls aged 11 to 15 years	4 years	General population	Self-reported PA estimated from the Past Year Leisure Physical Activity Scale	BMI	No association between past year PAL and BMI increases was observed
Wardle (2007) (UK)	2727 students aged 11-12 years at baseline	5 years	General population	Self-reported and teacher-reported number of physical education classes per week	BMI and waist circumference	Higher levels of physical education classes were associated with lower gains in adiposity in boys

PAL = physical activity level

PA = physical activity

BMI = body mass index

DEXA = dual-energy X-ray absorptiometry

Table II. Quality of the studies, as defined by the Downs and Black modified scale¹³.

Criteria ^a	Number of articles	
	Adequate	Inadequate
1. Is the hypothesis/aim/objective of the study clearly described?	24	0
2. Are the main outcomes to be measured clearly described in the Introduction or Methods section?	24	0
3. Are the characteristics of the patients included in the study clearly described?	24	0
4. Are the interventions of interest clearly described?	24	0
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?	6	18
6. Are the main findings of the study clearly described?	24	0
7. Does the study provide estimates of the random variability in the data for the main outcomes?	24	0
8. Have all important adverse events that may be a consequence of the intervention been reported?	-	-
9. Have the characteristics of patients lost to follow-up been described?	11	13
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	17	7
11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	8	16
12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?	10	14
13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?	-	-
14. Was an attempt made to blind study subjects to the intervention they have received?**	4	20
15. Was an attempt made to blind those measuring the main	4	20

outcomes of the intervention?**

16. If any of the results of the study were based on “data dredging”, was this made clear?	22	2
17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls?	20	4
18. Were the statistical tests used to assess the main outcomes appropriate?	21	3
19. Was compliance with the intervention/s reliable?**	11	13
20. Were the main outcome measures used accurate (valid and reliable)?	5	19
21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population?	21	3
22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time?	19	5
23. Were study subjects randomised to intervention groups?	-	-
24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?	-	-
25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	18	6
26. Were losses of patients to follow-up taken into account?	9	15
27. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?	3	21

^a Questions in grey are specific for experimental studies, and therefore, were not rated in the table.

** Observational studies were coded as zero (“unable to determine”).

ANEXOS

Anexo I – Questionário



UNIVERSIDADE FEDERAL DE PELOTAS
FACULDADE DE MEDICINA



ESTUDO LONGITUDINAL DAS CRIANÇAS
NASCIDAS EM 1993 NA CIDADE DE PELOTAS

QUESTIONÁRIO DO/A ADOLESCENTE

Número do questionário _____

EU GOSTARIA DE MEDIR A TUA PRESSÃO DUAS VEZES. AGORA E NO FIM DA ENTREVISTA			

1.	Pressão arterial sistólica (número maior)	_____	mm/Hg
2.	Pressão arterial diastólica (número menor)	_____	mm/Hg
AGORA VOU TE PERGUNTAR SOBRE OS TEUS ESTUDOS			
3.	Tu já estudaste em colégio alguma vez?	(0) não	(1) sim
SE ESTÁ ESTUDANDO, FAÇA AS PERGUNTAS DE 4 A 17			
4.	Como tu vais para o colégio: a pé, de ônibus, de carro, bicicleta?	(1) carro ou moto (2) ônibus (3) a pé (4) bicicleta () outro _____	
5.	Quanto tempo tu demoras até chegar no colégio?	_____ minutos	
6.	SE VAI DE BICICLETA: Tu vais pedalando ou de carona?	(1) pedalando	(2) de carona
7.	SE VAI DE ÔNIBUS: Quanto tempo tu caminhas até chegar na parada?	_____ minutos	
8.	SE VAI DE ÔNIBUS: Quanto tempo tu caminhas da parada até o colégio?	_____ minutos	
9.	Como tu voltas do colégio?	(1) carro ou moto (2) ônibus (3) a pé (4) bicicleta () outro _____	
10.	Quanto tempo tu demoras do colégio até em casa?	_____ minutos	
11.	SE VOLTA DE BICICLETA: Tu voltas pedalando ou de carona?	(1) pedalando	(2) de carona
12.	SE VOLTA DE ÔNIBUS: Quanto tempo tu caminhas até chegar na parada?	_____ minutos	
13.	SE VOLTA DE ÔNIBUS: Quanto tempo tu caminhas da parada até a tua casa ou até o lugar para onde vais depois da aula?	_____ minutos	
14.	Tu tens aula de Educação Física no colégio?	(0) não	(1) sim
15.	SE SIM: Tu participas das aulas ou és dispensado?	(1) participa	(2) dispensado
16.	SE PARTICIPA: Quantas vezes por semana tu tens aula de Educação Física?	____ vezas por semana	
17.	SE É DISPENSADO: Por que tu és dispensado? _____		

AGORA VOU PERGUNTAR SOBRE OUTRAS ATIVIDADES TUAS		
18. Tu assistes televisão?	(0) não (1) sim	
19. SE SIM: Quantas horas tu assistes televisão nos domingos?	____ horas ____ minutos	
20. SE SIM: Quantas horas tu assistes televisão em um dia de semana sem ser sábado e domingo?	____ horas ____ minutos	
21. Tu jogas videogame?	(0) não (1) sim	
22. SE SIM: Quantas horas tu jogas videogame nos domingos?	____ horas ____ minutos	
23. SE SIM: Quantas horas tu jogas videogame em um dia de semana sem ser sábado e domingo?	____ horas ____ minutos	
24. Tu usas computador?	(0) não (1) sim	
25. SE SIM: Quantas horas tu ficas no computador nos domingos?	____ horas ____ minutos	
26. SE SIM: Quantas horas tu ficas no computador em um dia de semana sem ser sábado e domingo?	____ horas ____ minutos	

27. Desde <DIA> da semana passada, tu praticaste alguma das atividades que vou dizer SEM CONTAR AS AULAS DE EDUCAÇÃO FÍSICA...		
	QUANTOS DIAS NA SEMANA?	QUANTO TEMPO CADA DIA?
a) futebol de sete, rua ou campo?	____	____ horas ____ minutos
b) futebol de salão (futsal)?	____	____ horas ____ minutos
c) atletismo?	____	____ horas ____ minutos
d) basquete?	____	____ horas ____ minutos
e) jazz, ballet, outras danças?	____	____ horas ____ minutos
f) ginástica olímpica, rítmica ou GRD?	____	____ horas ____ minutos
g) judô, karatê, capoeira, outras lutas?	____	____ horas ____ minutos
h) natação?	____	____ horas ____ minutos
i) vôlei?	____	____ horas ____ minutos
j) tênis, pádel?	____	____ horas ____ minutos
l) handebol?	____	____ horas ____ minutos
m) caçador?	____	____ horas ____ minutos
n) jogo de taco?	____	____ horas ____ minutos
o) outro esporte? _____	____	____ horas ____ minutos

28. Comparando com os teus amigos da mesma idade tua, tu fazes... (ler opções)
(1) mais exercício que eles (2) menos exercício que eles (3) a mesma quantidade que eles

29. SE ESTÁ ESTUDANDO: Sem contar as aulas de Educação Física, tu participas de alguma escolinha, time, dança ou ginástica no teu colégio? (só contar atividades com professor ou instrutor)

(0) não (1) sim

30. SE SIM: Quais?

Futebol (0) não (1) sim

Futsal (0) não (1) sim

Vôlei (0) não (1) sim

Basquete (0) não (1) sim

Handebol (0) não (1) sim

Danças (0) não (1) sim

Lutas (0) não (1) sim

Ginásticas (0) não (1) sim

Outra _____

31. Tu participas de alguma escolinha, time, dança ou ginástica sem ser em colégio? (só contar atividades com professor ou instrutor)

(0) não (1) sim

32. SE SIM: Quais?

Futebol (0) não (1) sim

Futsal (0) não (1) sim

Vôlei (0) não (1) sim

Basquete (0) não (1) sim

Handebol (0) não (1) sim

Danças (0) não (1) sim

Lutas (0) não (1) sim

Ginásticas (0) não (1) sim

Outra _____

AGORA EU GOSTARIA DE SABER SOBRE TEUS HÁBITOS ALIMENTARES NO ÚLTIMO ANO. PENSA AGORA SOBRE TEUS HÁBITOS ALIMENTARES DURANTE O ANO PASSADO. PENSA SOBRE QUANTAS VEZES TU COMESTE CADA UM DOS SEGUINTE ALIMENTOS.

ALIMENTO	≤ 1x/mês (0)	2-3x/mês (1)	1-2x/sem (2)	3-4x/sem (3)	5 + x/sem (4)	Escore
33. Hambúrguer, cheesesburger, Bauru						
34. Bife ou carne assada						
35. Frango frito						
36. Cachorro quente						
37. Presunto, embutidos						
38. Maionese comum						
39. Margarina ou manteiga						
40. Ovos						
41. Bacon ou lingüiça						
42. Queijo ou requeijão						
43. Leite Integral						
44. Batata frita						
45. Chips ou pipoca						
46. Sorvete ()						

47. Bolo, bolacha, pastéis, massas folhadas							
AGORA EU VOU DIZER OUTRA LISTA DE ALIMENTOS E GOSTARIA QUE TU ME DISSESSES QUANTAS VEZES POR DIA OU SEMANA TU COMESTE ESTES ALIMENTOS NESTE ÚLTIMO MÊS							
ALIMENTO	< 1x/sem (0)	1/sem (1)	2-3x/sem (2)	4-6x/sem (3)	Todo dia (4)	Escore	
48. Suco de laranja natural							
49. Fruta, sem contar suco							
50. Salada Verde							
51. Batata							
52. Feijão							
53. Outros vegetais							
54. Cereal Integral							
55. Pão Integral/Centeio							
56. Pão Branco							
57. Refrigerante							
58. Refrigerante dietético							
59. Doces, sobremesas							
AGORA VAMOS FALAR SOBRE OUTRAS COISAS DA TUA ALIMENTAÇÃO							
60. Tu tens o hábito de comer a gordura da carne?							(0) não (1) sim
61. Tu tens o hábito de comer a pele da galinha ou frango?							(0) não (1) sim
62. Desde <MÊS> do ano passado, tu fizeste algum tipo de regime para emagrecer?							(0) não (1) sim
63. SE SIM: Este regime foi dado por médico ou nutricionista?							(0) não (1) sim
64. SE FEZ REGIME NO ÚLTIMO ANO: E agora, estás fazendo regime?							(0) não (1) sim
65. Desde <MÊS> do ano passado, tu tomaste algum remédio para emagrecer?							(0) não (1) sim
66. Desde <DIA> do mês passado, tu tomaste alguma vitamina ou ferro?							(0) não (1) sim
67. Depois que o teu prato já está servido, tu costumas colocar mais sal na comida?							(0) não (1) sim
68. Qual dessas coisas tu usas mais seguido no pão, torrada ou bolacha? (ler opções)							
(1) manteiga (2) margarina (3) maionese (4) requeijão (5) patê (6) nenhum () outro							
69. Quando tomas refrigerante, qual tipo tomas? (ler opções)							
(1) diet/light (2) normal (3) os dois (4) não toma							
ESTÁGIOS DE TANNER							

70. Número para tamanho	(1)	(2)	(3)	(4)	(5)
71. Número para quantidade de pêlos	(1)	(2)	(3)	(4)	(5)
SOMENTE PARA AS MENINAS					
72. Tu já menstruaste?	(0) não (1) sim				
73. Que idade tu tinhas quando menstruaste pela primeira vez?	____ anos ____ meses				
74. Qual a data da tua última menstruação?	____ dias				
MENINAS E MENINOS					
AGORA EU GOSTARIA DE FAZER ALGUMAS MEDIDAS					
75. Peso (anotar roupas que o adolescente usava durante medida):	____, ____ kg				
<hr/>					
76. Altura	____, ____ cm				
77. Circunferência abdominal	____, ____ cm				
78. Circunferência do quadril	____, ____ cm				
79. Circunferência do braço	____, ____ cm				
80. Circunferência da coxa	____, ____ cm				
81. Dobra cutânea tricipital (braço)					
MEDIDA 1: ____ , ____ mm	MEDIDA 2: ____ , ____ mm	MEDIDA 3: ____ , ____ mm			
82. Dobra cutânea subescapular (costas)					
MEDIDA 1: ____ , ____ mm	MEDIDA 2: ____ , ____ mm	MEDIDA 3: ____ , ____ mm			
83. Pressão arterial sistólica (número maior)	____ mm/Hg				
84. Pressão arterial diastólica (número menor)	____ mm/Hg				
COLETAR A AMOSTRA DE SALIVA E DAR A DOSE					
85. HORA EXATA QUE O ADOLESCENTE INGERIU A DOSE	____	h	____	min	
AVISAR QUE ELE SERÁ PROCURADO NOVAMENTE DAQUI A 4 À 5H PARA TER A SALIVA COLETADA NOVAMENTE. A VISITA NÃO DEMORARÁ MAIS DO QUE 2MIN.					
LOCAL ONDE O ADOLESCENTE ESTARÁ DAQUI A 4 À 5H					
<hr/>					
ENTREGAR E EXPLICAR O FUNCIONAMENTO DOS APARELHOS					

**MUITO OBRIGADA POR TUA COLABORAÇÃO. FOI MUITO IMPORTANTE TU TERES PARTICIPADO
NESTA ETAPA DO ESTUDO. → AS PRÓXIMAS PERGUNTAS SÃO PARA VOCÊ**

86. Nome do entrevistador: _____ código ____

87. Data da entrevista: ____ / ____ / 20 ____

Anexo II - Preparação das doses de deutério



UNIVERSIDADE FEDERAL DE PELOTAS FACULDADE DE MEDICINA

***ESTUDO LONGITUDINAL DAS CRIANÇAS
NASCIDAS EM 1993 NA CIDADE DE PELOTAS***



D1 - Número de identificação do adolescente na coorte

/ /06

D2 - Data da preparação da dose

Rotular a garrafa e o microtubo com o número do adolescente (não esquecer de adicionar código referente a preparação da dose)

-se for preparar mais de uma dose ao mesmo tempo, lembrar de rotular as garrafas e microtubos
ANTES

Encher a garrafa com água mineral até a marca indicada na garrafa

Adicionar 2g de deutério utilizando a seringa de 1ml e o filtro (pode utilizar o mesmo filtro e seringa para, no máximo, 25 doses)

Agitar a garrafa com água + 2g de deutério por pelo menos 1minuto

Utilizar uma pipeta nova para retirar uma amostra da dose preparada e inserir no microtubo que já deve estar rotulado com o número de identificação

Todos os passos acima foram realizados?

Colocar a garrafa e um canudo na sacola plástica

Zerar as escalar

D3 - Peso da garrafa (cheia) + canudo + sacola plástica , g

ENTREGAR A SACOLA COM GARRAFA E CANUDO PARA ENTREVISTADOR
Entregar o algodão para coletar saliva rotulado com o número da criança, mais código referente à saliva
(número 2 – pré-dose ou 3 – pós-dose)

PROCEDIMENTOS APÓS O ADOLESCENTE TER INGERIDO A DOSE

D4 - Pesar garrafa (vazia) + canudo + sacola plástica , g

D5 - Peso do líquido ingerido pelo adolescente (D3 – D4) , g

Anexo III – Coleta de deutério



**UNIVERSIDADE FEDERAL DE PELOTAS
FACULDADE DE MEDICINA**

**ESTUDO LONGITUDINAL DAS CRIANÇAS
NASCIDAS EM 1993 NA CIDADE DE PELOTAS**



D6 – Número de identificação do adolescente na coorte

D7 – Hora da ingesta de deutério

hmin

D8 – Hora da coleta de saliva pós-dose

hmin

D9 - Quantidade dos líquidos ingeridos no período entre a dose e 5h:

1 latinha de refri = 350ml

1 refri pequeno = 290ml

1 copo de água = 200ml

TOTAL ml

Anexo IV - Preparação das doses de água duplamente marcada



**UNIVERSIDADE FEDERAL DE PELOTAS
FACULDADE DE MEDICINA**

**ESTUDO LONGITUDINAL DOS ADOLESCENTES
NASCIDOS EM 1993 NA CIDADE DE PELOTAS**



DLW1 - Número de identificação do adolescente na coorte

<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
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DLW2 - Data da preparação da dose

<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	/	<input type="text"/>	<input type="text"/>	/06
----------------------	----------------------	----------------------	----------------------	---	----------------------	----------------------	-----

Rotular a garrafa e o tubo com o número do adolescente

Peso do adolescente

<input type="text"/>	<input type="text"/>	,	<input type="text"/>	kg
----------------------	----------------------	---	----------------------	----

Dose de oxigênio 18 necessária (Peso em kg * 1.5 g/kg)

<input type="text"/>	<input type="text"/>	,	<input type="text"/>	<input type="text"/>	g
----------------------	----------------------	---	----------------------	----------------------	---

Dose de deutério necessária (Peso em kg * 0.075 g/kg)

<input type="text"/>	,	<input type="text"/>	<input type="text"/>	g
----------------------	---	----------------------	----------------------	---

Colocar a garrafa na balança. Tarar a balança.

Adicionar a quantidade de O18 na garrafa, utilizando uma seringa de 20ml e filtro.

Zerar a escala novamente.

Adicionar a quantidade de deutério necessária na garrafa, utilizando uma seringa de 1ml e filtro.

Completar a garrafa com água mineral.

Mexer o conteúdo da garrafa por pelo menos 1minuto.

Retirar uma amostra para o microtubo, utilizando uma pipeta.

Todos os passos acima foram realizados?

Colocar a garrafa e um canudo dentro da sacola plástica

 ,

DLW3 - Pesar tudo

g

Rotular os sete tubos universais que serão utilizados para o mesmo adolescente

Exemplo para o adolescente número 3456:

Tubo de pré-dose: 3456 – PDU

Tubo de pré-dose: 3456 – SAL

Dia da dose: 3456-4 (significa a amostra de saliva 4 horas pós-dose)

Dia 1: 3456 – 1

Dia 2: 3456 – 2

Dia 3: 3456 – 3

Dia 10: 3456 – 8

Dia 11: 3456 – 9

Dia 12: 3456 – 10

DLW4 - Pesar a garrafa (vazia) + canudo + sacola plástica, ao chegarem do campo

 , g

Anexo V - Coleta de água duplamente marcada



**UNIVERSIDADE FEDERAL DE PELOTAS
FACULDADE DE MEDICINA**



COLETA DE ÁGUA DUPLAMENTE MARCADA

DLW5 - Número de identificação da criança na coorte

DLW6 - Hora de coleta da amostra de urina pré-dose

min _____ h _____

DLW7 - Hora de coleta da amostra de saliva pré-dose

_____ min _____ h _____

⇒ DAR A DOSE PARA A CRIANÇA

DLW8 - Data da ingestão da dose (dia zero) / /07

DLW9 - Hora de ingestão da dose

h min

COMBINAR COM O ADOLESCENTE A COLETA DE SALIVA DAQUI A 5 HORAS
DLW10 - Anote aqui a hora prevista para a coleta de saliva

arrive h min

Essa folha é para ser usada na coleta de saliva 5horas após a ingestão da dose

Perguntar ao adolescente a quantidade de líquidos que ele tomou desde a hora em que tomou a água (ou seja, nas últimas 4h). (você pode anotar em quantidade de copos d'água/refrigerante/sucos, etc.)

DLW11 – quantidade total (em ml) (*deixar em branco*)

TOTAL _____

A T E N Ç Ã O!!!!

O ADOLESCENTE NÃO DEVE TER ESCOVADO OS DENTES NEM INGERIDO NADA NOS ÚLTIMOS 10 MINUTOS (LÍQUIDOS E COMIDA)

DLW12 - Hora da coleta de saliva pós-dose (+ 5 horas)

_____ h _____ min

FICHA PARA ANOTAR OS DIAS E HORÁRIOS PARA COLETA DE URINA

DLW13 - Hora de coleta da amostra de urina (dia 1 ____ /____ /07)

_____ h _____ min

DLW14 - Hora de coleta da amostra de urina (dia 2 ____ /____ /07)

_____ h _____ min

DLW15 - Hora de coleta da amostra de urina (dia 3 ____ /____ /07)

_____ h _____ min

DLW16 - Hora de coleta da amostra de urina (dia 8 ____ /____ /07)

_____ h _____ min

DLW17 - Hora de coleta da amostra de urina (dia 9 ____ /____ /07)

_____ h _____ min

DLW18 - Hora de coleta da amostra de urina (dia 10 ____ /____ /07)

_____ h _____ min

Anexo VI - Pranchas de Tanner

Figura 1. Prancha I - masculina . **Observar os pêlos**

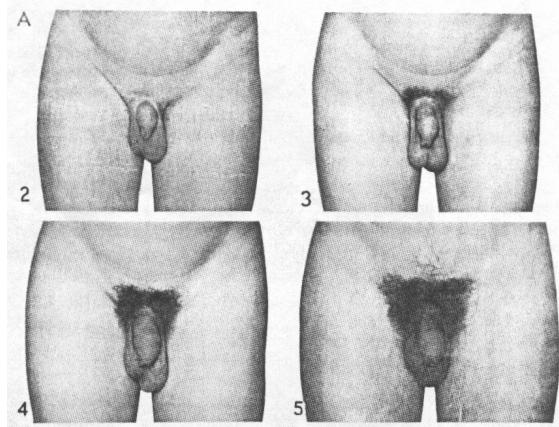


Figura 2. Prancha II - masculina. **Observar o tamanho e a forma do saco escrotal e do pênis**

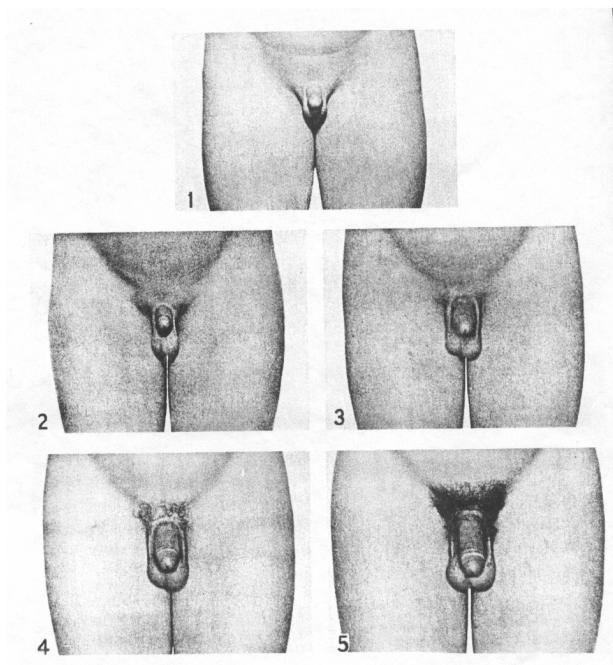


Figura 1. Prancha I - Feminina. **Observar os pêlos.**

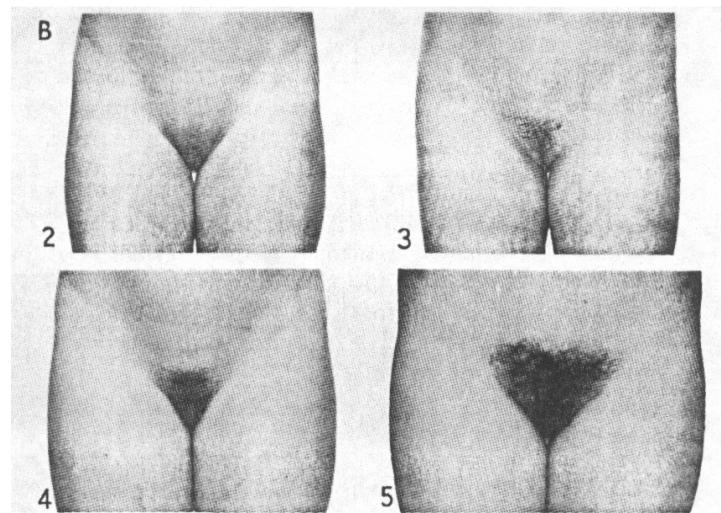


Figura 2. Prancha II - Feminina. **Observar o tamanho e a forma das mamas (seios)**

