UNIVERSIDADE FEDERAL DE PELOTAS
FACULDADE DE MEDICINA
DEPARTAMENTO DE MEDICINA SOCIAL
PROGRAMA DE PÓS-GRADUAÇÃO EM EPIDEMIOLOGIA

**Tese de Doutorado**

# Epidemiologia da hemoglobina glicada (HbA1c) em duas coortes de nascimentos - Pelotas/RS

Romina Buffarini

Pelotas - RS
Março, 2017

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Orientadora: Prof.ª Dr.ª Maria Cecília Formoso Assunção

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Romina Buffarini

# Epidemiologia da hemoglobina glicada (HbA1c) em duas coortes de nascimentos - Pelotas/RS

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*"Por vezes sentimos que aquilo que fazemos não é senão uma gota de água no mar. Mas o mar seria menor se lhe faltasse uma gota". (Madre Teresa de Calcuta)*

A meus amores, Fernando e Camus

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Ao meu grande companheiro, Fernando, pela força que sempre me deu para percorrer a jornada. Obrigada pelo incentivo e ajuda para encarar os novos desafios. Tivesse sido impossível terminar o doutorado sem seu apoio e cumplicidade no dia a dia. Amor, conseguimos!

Ao meu pequeno grande amor, à luz dos meu olhos, meu bebê Camus, que veio ao mundo no meio desse doutorado para deixá-lo ainda mais intenso.

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Resumo

BUFFARINI, Romina. **Epidemiologia da hemoglobina glicada (HbA1c) em duas coortes de nascimentos - Pelotas/RS.** 2016. Tese (Doutorado). Programa de Pós-Graduação em Epidemiologia. Universidade Federal de Pelotas (UFPel).

A hemoglobina glicada (HbA1c) é o melhor marcador de controle glicêmico em longo prazo em pacientes diabéticos e uma importante ferramenta na triagem e diagnóstico de diabetes na população em geral. O teste de HbA1c oferece algumas vantagens em comparação com outros indicadores glicêmicos: pode ser realizado a qualquer hora do dia, independentemente do tempo de jejum, e, é relativamente barato. Em estudos epidemiológicos, a HbA1c foi associada com a incidência de diabetes e doenças cardiovasculares (CVD) e com mortalidade por todas as causas em populações livres de doença. Portanto, a HbA1c pode ser utilizada para examinar o risco de doença associado à hiperglicemia em populações saudáveis. Esta tese teve por objetivo descrever a epidemiologia da HbA1c (distribuição e fatores associados) e avaliar a associação de padrões de crescimento com a HbA1c e outros marcadores de risco cardiometabólico em adolescentes e adultos jovens. Uma melhor compreensão dessas relações pode ajudar a identificar grupos da população com maior risco de doenças cardiometabólicas. A tese inclui três artigos. No primeiro, realizamos uma revisão sistemática da literatura que avalia a relação entre crescimento (mudanças de peso, altura ou índice de massa corporal (IMC) durante a infância, adolescência e indicadores de metabolismo glicêmico e da insulina na idade adulta (glicemia de jejum, glicemia de duas horas após o teste de tolerância oral à glicose, hiperglicemia, HOMA-IR). Foram identificadas algumas inconsistências nas definições de crescimento e nos intervalos de idade avaliados. A evidência mais consistente foi a relação entre ganho acelerado de peso ou IMC depois dos dois anos de vida e alterações no metabolismo da glicemia e de insulina na idade adulta. No segundo artigo, descrevemos a distribuição dos níveis de HbA1c em indivíduos em adolescentes e adultos (18 e 30 anos, respectivamente) participantes das coortes de nascimentos de Pelotas de 1993 e 1982, de acordo com fatores precoces e contemporâneos. A distribuição da HbA1c foi aproximadamente normal em ambas as coortes. Os níveis médios de HbA1c foram mais elevados nos indivíduos com cor de pele preta/morena em comparação com aqueles de pele branca em ambas as coortes. A história familiar de diabetes esteve associada com uma maior média de HbA1c em adultos, enquanto o baixo comprimento para idade no primeiro ano de vida apresentou uma relação inversa com o desfecho em adolescentes. Nenhum outro fator precoce e contemporâneo foi associado aos níveis de HbA1c em adultos ou adolescentes. No terceiro artigo, avaliamos a associação entre padrões de crescimento (crescimento linear e ganho de peso relativo) desde o nascimento até a adolescência e marcadores de risco cardiometabólico aos 18 anos, na Coorte de Nascimento de Pelotas de 1993. Os desfechos avaliados foram: glicemia ao acaso, HbA1c, proteína C reativa, colesterol total, LDL-C, HDL-C, triglicerídeos, pressão arterial sistólica e diastólica, IMC e circunferência da cintura. O estudo mostrou que o ganho de peso acelerado, especialmente depois dos dois primeiros anos de vida, está positivamente associado a

vários marcadores de risco cardiometabólico em adolescentes. Mais estudos são necessários para esclarecer a evidência sobre crescimento linear e fatores cardiometabólicos. Em geral, os nossos resultados sugerem que a prevenção do ganho de peso excessivo após os dois primeiros anos pode ser importante na redução do risco cardiometabólico na vida adulta.

Palavras-chave: Hemoglobina glicada; Alterações no metabolismo glicêmico; Marcadores de risco cardiometabólico; Adolescentes; Adultos jovens; Estudos de Coortes; Epidemiologia.

Abstract

BUFFARINI, Romina. **Epidemiology of glycated hemoglobin (HbA1c) in two birth cohorts - Pelotas/RS.** 2016. Thesis (Doctoral Thesis). Postgraduate Program in Epidemiology. Federal University of Pelotas (UFPel).

Glycated haemoglobin (HbA1c) is the best index for long-term glucose control in diabetic patients and a screening tool for detecting diabetes in general population. The $HbA_{1c}$ measurement offers some advantages compared with other glycemic indicators. It can be performed at any time of the day, irrespective of fasting or feeding, and it is relatively cheap. In epidemiological studies, $HbA_{1c}$ has been found to be associated with the incidence of diabetes and cardiovascular diseases (CVD), and all-cause mortality in populations free of disease. Therefore, HbA1c is used to examine the disease risk associated to hyperglycaemia in healthy populations. This thesis aimed to describe the epidemiology of HbA1c (distribution and associated factors) and to evaluate the association of growth patterns with cardiometabolic risk markers in adolescents and young adults. A better understanding of the these relations may help to identify groups of the population with increased risk of cardiometabolic diseases. The thesis includes three papers. In the first paper, we systematically reviewed literature assessing the relationship between growth (change in weight, height or BMI) during infancy, childhood and adolescence and indicators of glucose and insulin metabolism in adulthood (fasting glycemia, 2 hours glycemia after an Oral Glucose Tolerance Test, hyperglycemia, HOMA-IR). Some inconsistencies in growth definitions and age intervals were identified. The most consistent evidence was documented for rapid weight or body mass index (BMI) gain from childhood onwards and a worse glucose and insulin metabolism profile at adult age. In the second paper, we described the distribution of HbA1c levels in 18 and 30 years old individuals according to early-life and contemporary factors in the 1993 and 1982 Pelotas Birth Cohorts. The distribution of the HbA1c was approximately normal. HbA1c mean levels were significantly higher in individuals self-reported as black/brown skin color compared to those self-reported as white in both cohorts. Parental history of diabetes was associated with higher HbA1c mean in adults, while stunting at one year old presented an inverse relation with the outcome in adolescents. No other early and contemporary factors were associated with HbA1c levels in adults or adolescents. In the third paper, we evaluated the effect of growth trajectories (linear growth and relative weight gain) from birth to adolescence on cardiometabolic risk markers levels at age 18 years in the 1993 Pelotas Birth Cohort. The outcomes were random glucose, HbA1c, C-reactive protein, total cholesterol, LDL-C, HDL-C, triglycerides, systolic and diastolic blood pressure, BMI and waist circumference. Our study showed that rapid weight gain from childhood onwards is positively associated with several markers of cardiometabolic risk in adolescents. Evidence on linear growth needs further analyses. Overall, our results suggest that prevention of excessive weight gain after the two first postnatal years might be important in reducing cardiometabolic risk later in life.

Key-words: Glycated hemoglobin; Cardiometabolic risk markers; Adolescents; Young adults; Cohort Studies; Epidemiology

## Sumário

# APRESENTAÇÃO

Esta tese, elaborada conforme o os moldes regimentais adotados pelo Programa de Pós-graduação em Epidemiologia (PPGE) da Universidade Federal de Pelotas, está composta por cinco seções.

Inicialmente, é apresentado o projeto de pesquisa, defendido em setembro de 2014, incorporando as sugestões e correções propostas pela banca examinadora. A seguir, é apresentada uma seção descrevendo modificações no projeto, realizadas posteriormente. Após, é apresentada um relato da experiência de trabalho de campo juntamente com o protocolo de coleta sanguínea e dosagem da hemoglobina glicada, desfecho de interesse do projeto. Posteriormente, são apresentados os três artigos produzidos ao longo do período de doutoramento e, finalmente, um comunicado à imprensa sintetizando os principais achados do estudo.

Os artigos são apresentados conforme o formato requerido pelas revistas aos quais foram ou serão submetidos. O primeiro, intitulado *"Growth patterns during infancy, childhood and adolescence and glucose and insulin metabolism in adulthood: a systematic review*", está em revisão na revista *Preventive Medicine*. O segundo artigo, *"Distribution of glycated haemoglobin according to early-life and contemporary characteristics in adolescents and adults without diabetes: the 1982 and 1993 Pelotas birth cohorts"*, foi publicado na revista *Plos One*, em Setembro de 2016. O terceiro manuscrito (*"Growth across life course and cardiometabolic risk markers in 18 years old adolescents: the 1993 Pelotas Birth Cohort"*), será submetido à revista *American Journal of Epidemiology*.

# PROJETO DE PESQUISA

UNIVERSIDADE FEDERAL DE PELOTAS
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**Projeto de Tese**

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Pelotas - RS
Setembro, 2014

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Doutoranda: Romina Buffarini
Orientadora: Prof.ª Dr.ª Maria Cecília Formoso Assunção
Coorientadora: Dr.ª María Clara Restrepo Méndez

Pelotas - RS
Setembro, 2014

# Resumo

Níveis elevados de glicose no sangue estão associados com aumento de risco para doença cardiovascular, mesmo em pessoas não diabéticas. A hemoglobina glicada (HbA1c), produto de uma reação não enzimática entre a hemoglobina e a glicose, é um marcador de hiperglicemia. Ela indica a média de glicemia no período de oito a doze semanas anteriores e está relacionada positivamente à concentração de glicose no sangue. Ela também reflete o estresse oxidativo e a glicação de tecidos, incluindo o vascular, sendo um marcador de risco para o desenvolvimento de complicações micro e macrovasculares no diabetes mellitus (DM). Além disso, estudos clínicos e epidemiologicos apontam que a HbA1c é um fator de risco independente para doenças cardiovasculares em indivíduos não diabéticos.

Concentrações dentro dos limites considerados normais (<6,5%) estão positivamente associadas com risco de doença cardiovascular, aterosclerose e morte. Porém, pouco se sabe sobre a distribuição dos níveis séricos de HbA1c em adolescentes e adultos, assim como os fatores precoces e contemporâneos que afetam os níveis de HbA1c.

Com este estudo, pretende-se descrever a epidemiologia da HbA1c e fatores independentes, precoces e contemporâneos, associados aos 18 e 30 anos em indivíduos pertencentes às Coorte de Nascimentos de 1982 e 1993 de Pelotas – RS, Brasil. Além disso pretende-se avaliar se os crescimentos intra e extrauterino (na infância e adolescência) têm influência no metabolismo da glicemia e, portanto, repercutindo nos níveis de HbA1c em adolescentes e adultos.

# Artigos planejados

**Artigo 1**

- Revisão sistemática: Padrões de crescimento e metabolismo da glicose

Objetivo: Realizar uma revisão sistemática de estudos que tenham avaliado a associação entre ganho de peso, ganho de índice de massa corporal e/ou ganho de estatura acelerados na infância e/ou adolescência e o metabolismo da glicose, em idades posteriores, em indivíduos sem diagnóstico de diabetes mellitus (DM). Os desfechos a serem avaliados serão marcadores de hiperglicemia: concentração plasmática de glicose em jejum, concentração plasmática de glicose depois do teste oral de tolerância à glicose, níveis de HbA1c.

**Artigo 2**

- Epidemiologia da hemoglobina glicada em adolescentes e adultos: Coortes de Nascimentos de 1993 e 1982 de Pelotas.

Objetivo: Descrever, transversalmente, a distribuição de hemoglobina glicada (HbA1c) segundo características genéticas, demográficas, socioeconômicas, de estilo de vida e de composição corporal, com ênfase nas medidas de adiposidade central aos 18 e 30 anos (Coorte de Nascimentos de 1993 e 1982, Pelotas, RS, Brasil).

**Artigo 3**

- Crescimento e hemoglobina glicada em adolescentes: Coorte de Nascimentos de 1993 de Pelotas.

Objetivo: Avaliar o tamanho ao nascer (peso e comprimento), crescimento linear, ganho de peso relativo à altura) durante a infância e adolescência em relação aos níveis de HbA1c medida aos 18 anos na Coorte de Nascimentos de 1993.

**Artigo 4**

- Trajetória de hemoglobina glicada dos 18 aos 30 anos: importância de fatores precoces e contemporâneos.

Objetivo: Avaliar a trajetória de hemoglobina glicada dos 18 aos 30 anos e fatores precoces e contemporâneos associados em uma subamostra de indivíduos do sexo masculino pertencentes à Coorte de Nascimentos de 1982 de Pelotas.

# Abreviaturas e definições de termos

| | |
|---|---|
| HbA1c | Hemoglobina glicada |
| BPN | Baixo Peso ao Nascer – peso ao nascer <2.500 gramas |
| CC | Circunferência da Cintura |
| DCV | Doenças Cardiovasculares |
| DM | Diabetes Mellitus |
| DCNT | Doenças Crônicas Não Transmissíveis |
| HAS | Hipertensão Arterial Sistêmica |
| HDL | Colesterol do tipo HDL (lipoproteína de alta densidade) |
| IMC | Índice de Massa Corporal |
| OMS | Organização Mundial da Saúde |
| IP | Índice Ponderal (peso ao nascer/ (comprimento ao nascer)$^3$) |
| PN | Peso ao Nascer |
| RCQ | Razão Cintura/ Quadril |
| IG | Idade Gestacional |

# Introdução

Atualmente, as doenças crônicas não transmissíveis (DCNT) são responsáveis por elevada carga de morbimortalidade no Brasil e na maioria dos países do mundo (1, 2), sendo que a maior proporção de mortes por DCNT é causada por doenças cardiovasculares (DCV) (3). Com base em dados da Organização Mundial da Saúde (OMS), a mortalidade atribuível às DCV em adultos de 30 a 70 anos no Brasil, em 2008, foi de 248 por 100.000 pessoas (3). Embora a mortalidade por DCV esteja diminuindo, estas continuam a ser responsáveis por um número importante de mortes, tanto no Brasil quanto em outros países do mundo (2, 4). Por outro lado, há um crescimento na prevalência de hipertensão arterial sistêmica (HAS) e diabetes mellitus (DM), o que ameaça o decréscimo das DCNT (2).

Além de desempenhar um papel direto nas complicações crônicas do DM, os níveis elevados de glicose no sangue estão associados ao excesso de risco para DCV e morte, mesmo em pessoas não diabéticas (3, 5). A hiperglicemia está entre os cinco fatores de risco mundiais mais importantes para a mortalidade, sendo responsável por, aproximadamente, um terço das mortes por doença cardíaca isquêmica e acidentes cerebrovasculares (6).

A hemoglobina glicada (HbA1c) elevada é um indicador do estado de hiperglicemia e tem sido associada com a incidência de eventos cardiovasculares, DM e morte por todas as causas (7-9). Este marcador apresenta estabilidade ao longo do tempo (10) e menor variabilidade intraindividual comparada com outros indicadores, como a glicemia em jejum. Além disso, o teste para medir os níveis de HbA1c no sangue é relativamente barato e não requer tempo de jejum (11, 12). Desta forma, a HbA1c é considerada uma medida muito útil para examinar o risco consequente à hiperglicemia em estudos populacionais (13).

Sendo a HbA1c um marcador hiperglicêmico associado a diversas doenças crônicas, torna-se importante avaliar a sua distribuição, assim como fatores precoces e contemporâneos associados em população adolescente e adulta sem presença de doenças crônicas.

As Coortes de Nascimentos de Pelotas, com informações sociodemográficas, antropométricas e de condições de saúde ao longo da vida dos participantes, fornecem uma fonte rica de dados para investigar a relação entre fatores precoces e contemporâneos e a HbA1c em adolescentes e adultos jovens.

# Marco teórico

A seguir, serão descritos alguns fundamentos em relação aos principais tópicos do estudo. Inicialmente, serão abordadas algumas considerações sobre a HbA1c, logo será descrito o papel da HbA1c como fator de risco para DCV e mortalidade e, finalmente, será apresentada a teoria da origem fetal das doenças do adulto.

Para a abordagem destes tópicos, foram realizadas buscas da literatura não sistemáticas, incluindo estudos considerados clássicos na epidemiologia do ciclo vital.

## Hemoglobina glicada: aspectos gerais

O termo genérico "hemoglobina glicada" refere-se a um conjunto de substâncias formadas com base em reações entre a hemoglobina A (HbA) e alguns açúcares (14). A HbA é a forma principal e nativa da hemoglobina, sendo que a HbA0 é o principal componente da HbA. Na prática, esta corresponde à chamada fração não glicada da HbA. Por outro lado, a HbA1c, também denominada apenas como A1c, é formada por um processo não enzimático, que se dá através da ligação da glicose com a hemoglobina A. A hemoglobina glicada está constituída por diferentes subtipos, cromatograficamente distintos, tais como HbA1a1, HbA1a2, HbA1b e HbA1c. A A1c é a fração da hemoglobina A1 onde está situada a ligação estável e irreversível do terminal valina da cadeia beta com a glicose, constituindo a hemoglobina glicada propriamente dita (Figura 1.) (14). Esta reação, aumentada na presença de hiperglicemia, causa alterações estruturais e funcionais nos tecidos, que contribuem para o desenvolvimento ou progressão de complicações macro e microvasculares, sobretudo associadas ao DM (15, 16). Portanto, a HbA1c é a única fração que deve ser mensurada e utilizada como medida do risco para complicações.

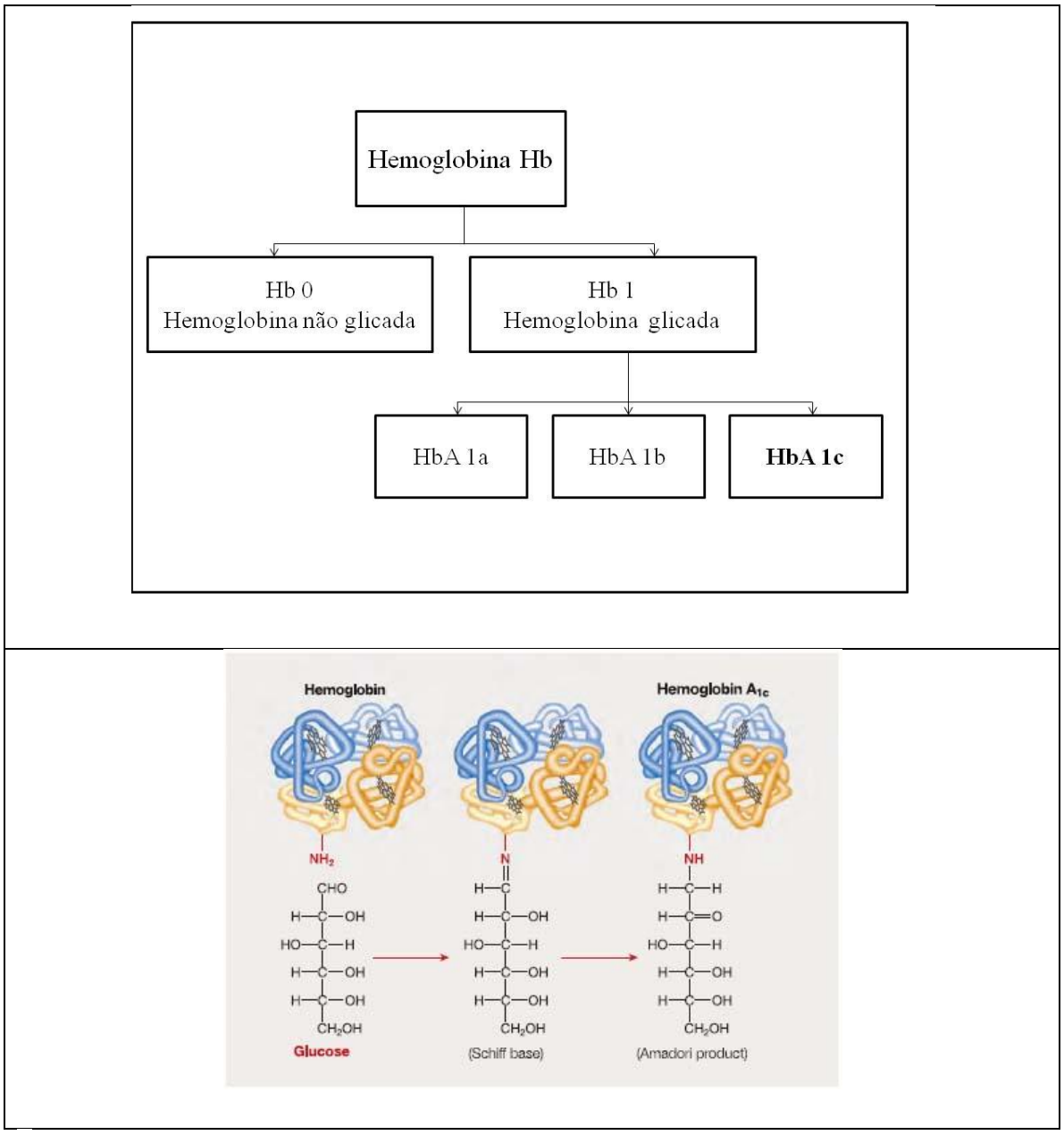


Figura 1. (a) Diferentes frações da hemoglobina. Adaptada de Netto, 2009 (14). (b) Formação de hemoglobina glicada A1c (Marchetti, P: http://www.medicographia.com) (17). HbA1c é formada a partir da ligação do grupo aldeído da glicose ao grupo amino-terminal da valina da cadeia de hemoglobina.

O nível sanguíneo de HbA1c é influenciado pelo ciclo de vida dos eritrócitos e pela permeabilidade à glicose (18, 19). Ele é um indicador de glicemia mais estável do que a glicemia de jejum (12), uma vez que diferenças na HbA1c refletem uma média

elevada de glicemia nos três meses precedentes (20), sendo que as glicemias mais recentes têm um impacto maior em relação às anteriores. Apesar de a glicação da hemoglobina ocorrer ao longo de todo o período de vida do eritrócito (120 dias aproximadamente), a glicemia mais recente é a que mais influencia o valor de HbA1c. Estudos clínicos sugerem que cerca de 50% da HbA1c corresponde àquela formada no mês precedente ao teste, 25% no mês anterior a este e os 25% remanescentes, no terceiro ou quarto meses antes do exame (21).

Adicionalmente, os níveis de HbA1c podem sofrer alterações em situações nas quais existe um intercâmbio anormal de eritrócitos. Níveis muito baixos são observados na presença de alterações no metabolismo sanguíneo, anemia hemolíticas, transfusões sanguíneas, em pacientes com doença renal crônica em diálise, portadores de doença hepática, deficiências nutricionais de ácido fólico, vitamina B6 e B12 e hipertireoidismo (22, 23). Por outro lado, gravidez, deficiência nutricional de ferro, insuficiência renal e hiperuricemia podem aumentar os níveis de HbA1c (8, 14).

Diferentes métodos laboratoriais podem medir diferentes frações de hemoglobina glicada, por exemplo: HbA1b, HbA1c ou HbA1 total. Por este motivo, foi criado o Programa Nacional de Padronização da Glicohemoglobina (*National Glycohemoglobin Standardization Program*), um comitê especial para padronização das determinações do teste de HbA1c. Este programa apresenta uma lista de testes e laboratórios aprovados, tendo como referência o desempenho analítico do método utilizado no Ensaio Clínico Randomizado de Controle e Complicações do Diabetes (Diabetes Control and Complications Trial). No Brasil, o Grupo Interdisciplinar para Padronização da Hemoglobina Glicada - A1C, pertencente à Sociedade Brasileira de Diabetes, recomenda o uso de métodos certificados pelo Programa Nacional de Padronização da Glicohemoglobina (Netto, 2009).

Os resultados dos testes podem ser apresentados em duas unidades de medida: percentagem (g de HbA1c/g hemoglobina), adotado pelo Programa Nacional de Padronização da Glicohemoglobina, e mmol HbA1c/mol hemoglobina, recomendado pela Federação Internacional de Química Clínica (*International Federation of Clinical Chemistry*) (24, 25). Na literatura científica, a unidade mais comumente utilizada na literatura é a percentagem (g de HbA1c/g hemoglobina).

## Hemoglobina glicada: utilidade clínica e utilidade epidemiológica

### Papel no controle e diagnóstico do Diabetes Mellitus

Historicamente, a HbA1c foi associada ao DM, sendo considerada, em estudos clínicos, o melhor indicador para controle metabólico em pacientes com esta doença, uma vez que está associada a complicações microvasculares, tais como retinopatia e neuropatia (20).

Em 2008, o Comitê Internacional (*The International Expert Committee*), formado por membros da Associação Americana de Diabetes, Associação Europeia para o estudo do Diabetes e Federação Internacional de Diabetes, recomendou o uso da HbA1c como método alternativo à glicose plasmática no diagnóstico de DM na população geral (26). O ponto de corte estabelecido para diagnóstico de DM é de valores iguais ou superiores a 6,5% (27, 28). Porém, não há consenso sobre quais seriam os valores normais. Associações de diabetes de distintos países propõem a categoria de "risco aumentado de diabetes" (também utilizam o termo pré-diabetes) com diferentes limites (Tabela 1). Por exemplo, a Associação Canadense de Diabetes classifica como pré-diabéticos os indivíduos com valores entre 6,0 e 6,4% e reconhece que valores acima de 5,5% representam risco para complicações cardiovasculares (28). Por outro lado, existe literatura mostrando que indivíduos com níveis de HbA1c abaixo de 6,0% ainda podem estar em risco, dependendo da presença de outros fatores de risco concomitantes, como obesidade e história familiar de DM (29). De modo geral, valores abaixo de 6,5% são considerados como concentração dentro dos limites normais.

Posteriormente, a partir dos resultados de estudos mostrando a hiperglicemia como marcador de risco para desenvolvimento das DCV na população geral, a HbA1c começou a ser vista como uma valiosa ferramenta para avaliar hiperglicemia em estudos populacionais (30).

Tabela 1. Classificação da hemoglobina glicada para o diagnóstico de DM.

| Classificação | HbAc1 (ADA) | (Canadá) |
|---|---|---|
| Normal | <5,7% | - |
| Pré-diabetes | 5,7 a 6,4% | 6,0% a 6,4% |
| Diabetes | ≥6,5% | ≥6,5% |

Adaptado da Associação Americana de Diabetes, 2014 e Associação Canadense de Diabetes, 2013 (27, 28).

**Papel da HbA1c como marcador de risco para doenças cardiovasculares, diabetes mellitus e mortalidade**

A dosagem de HbA1c apresenta diversas vantagens em relações a outros indicadores de hiperglicemia. Além disso, foi observado que o risco de ter placas ateroscleróticas aumenta conforme aumentam os níveis de HbA1c em indivíduos sem diagnóstico de DM (31, 32). Em estudos epidemiológicos, assume-se que uma única medida de HbA1c é um índice válido de controle glicêmico por um tempo muito maior do que aquele predito pelo ciclo de vida média dos eritrócitos – de 120 dias (13). Vitelli et al. sugerem que, com o passar do tempo, episódios repetidos de leves aumentos de glicose em indivíduos não diabéticos podem produzir o mesmo dano fisiológico que níveis de hiperglicemia observados no DM (31).

Em população adulta e idosa não diabética, vários estudos têm mostrado uma relação direta entre níveis de HbA1c e incidência de DCV, independentemente da presença de outros fatores de risco (7, 33-36). Estudos prospectivos que avaliaram adultos e idosos indicaram que níveis entre 5,5% e 6% representam alto risco para desenvolvimento de DM (37, 38) e de DCV, independentemente da presença de diabetes (8). Uma coorte de quatro comunidades norte americanas, com indivíduos entre 45 e 64 anos, revelou que níveis de HbA1c dentro da categoria considerada normal (entre 4,6 e 6,0%) foram associados com presença de DCV (39).

Resultados do estudo EPIC (*European Prospective Investigation into Cancer and Nutrition*), realizado em vários países da Europa, mostraram que cada aumento de 1% na

HbA1c leva a um aumento de 20 a 30% no risco de eventos cardiovasculares e de mortalidade por todas as causas, em ambos os sexos, independente da presença de diabetes (7). Em mulheres não diabéticas, a HbA1c tem se mostrado como melhor preditor de DCV e mortalidade por DCV em comparação à glicose de jejum ou pós-prandial (34).

Algumas pesquisas têm sugerido uma associação com curva em forma de "J" ou "U" entre níveis de HbA1c e mortalidade por todas as causas na população geral (sem diagnóstico de DM) (40, 41). Aggarwal et al. observaram que níveis de HbA1c abaixo de 5,0% foram associados a um aumento de risco de mortalidade por todas as causas e câncer, porém a etiologia desta relação não é clara (42).

## Teorias sobre a origem fetal das doenças crônicas do adulto

No início da década de 90, Barker e colaboradores propuseram a teoria da origem do desenvolvimento das doenças do adulto, também conhecida como "hipótese de Barker". A teoria sugere que a nutrição deficiente durante a gestação e infância precoce (sobretudo no primeiro ano de vida) originaria uma adaptação metabólica ou endócrina permanente, alterando a estrutura e função do corpo e, desta forma, aumentando o risco de desenvolvimento de DCV e outras doenças associadas, como HAS e DM, na vida adulta (43-45).

Baseado na teoria da "programação fetal", o conceito do "fenótipo econômico" ou “fenótipo poupador” (*thrifty phenotype*) sugere que uma adaptação fisiológica vantajosa em determinado momento da vida intrauterina pode se tornar uma desvantagem para o indivíduo quando exposto a um ambiente diferente - com grande aporte calórico - depois do nascimento (46, 47). A Figura 2 apresenta o mecanismo proposto nesta teoria. Por exemplo, a desnutrição durante determinados períodos comprometeria o desenvolvimento das células beta e a resposta à insulina, sendo a causa das alterações no metabolismo da glicose. Ao longo do ciclo vital, o risco individual de doença seria determinado pela interação de genes de susceptibilidade à doença, fatores sociodemográficos e comportamentais (45).

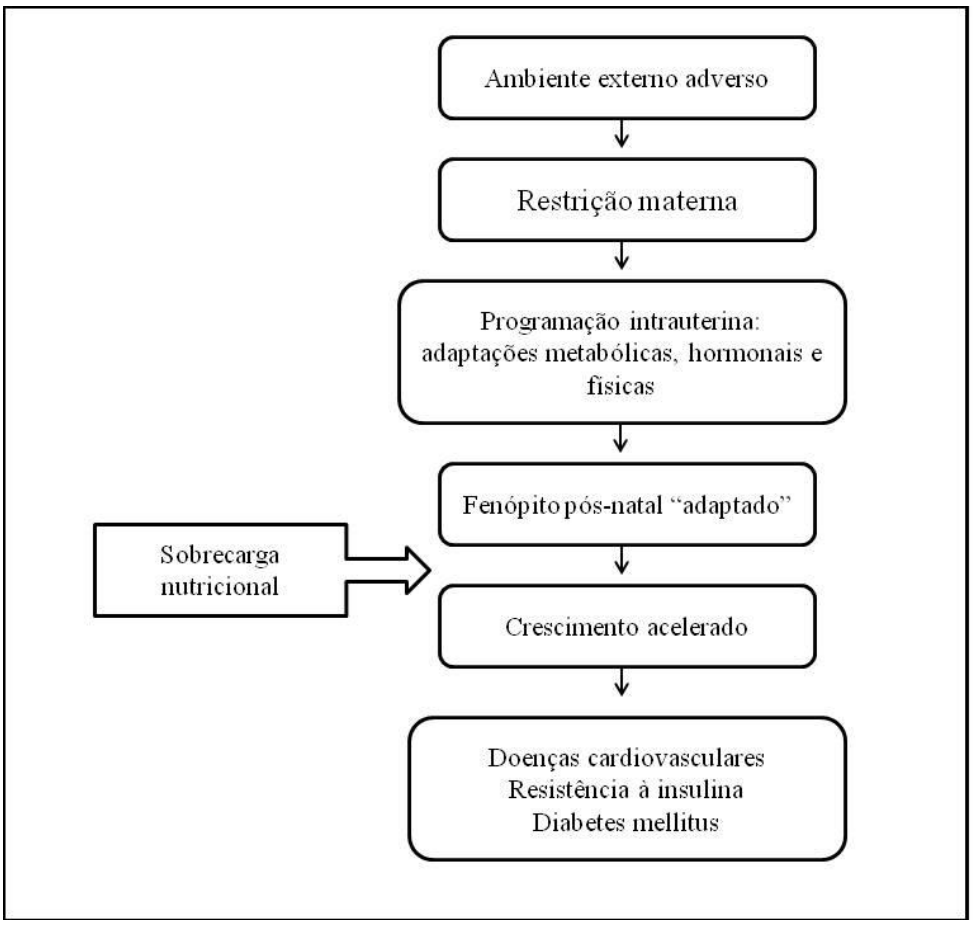


Figura 2. Modelo da teoria do fenótipo econômico. Adaptado de Leon, 2004 (47).

Desde então, estudos longitudinais realizados em diversas populações, principalmente coortes históricas em países de alta renda, mostraram resultados concordantes com esta teoria (48-51). Nestes estudos, o baixo peso ao nascer (BPN) e a subnutrição (déficit de peso/idade, altura/idade e/ou peso/altura) nos dois primeiros anos de vida se mostraram fatores de risco para DCV, DM e outras alterações no metabolismo lipídico e glicêmico. Contudo, estudos mais atuais sugerem que, entre aqueles indivíduos que apresentaram uma alta taxa de aumento de peso ou IMC durante a infância, os níveis de risco são ainda maiores (49, 52-58).

Depois dos dois anos de idade, aproximadamente, o IMC das crianças apresenta uma diminuição, atingindo o seu valor mínimo em torno dos seis anos, antes de aumentar novamente - fenômeno esse chamado de rebote de adiposidade. Na coorte de Helsinki foi observado que quanto menor a idade de ocorrência deste fenômeno, maior a incidência de

DM na vida adulta, indicando a importância da velocidade de ganho de peso no risco de desenvolver doenças posteriormente. Dados desta mesma coorte mostram, também, que o rebote da adiposidade em idades mais precoces foi associado ao menor ganho de peso no primeiro ano de vida, e, consequente, menor IMC com um ano de idade (59). Em concordância, outro estudo com dados da mesma coorte mostrou que BPN, baixo peso para idade aos dois anos e rápido ganho de IMC depois desta idade estão associados com o desenvolvimento de resistência à insulina na vida adulta (60). Em situações nas quais houve uma restrição de crescimento, este período é seguido por um crescimento rápido, acima dos padrões normais de crescimento para determinada idade. Este fenômeno é conhecido como crescimento compensatório e está associado com alterações hormonais e metabólicas persistentes. Estas alterações aumentam a demanda sobre a capacidade funcional inicialmente reduzida no período de restrição (61). Sendo assim, este processo apresenta vantagens e desvantagens em curto e longo prazos e é descrito como o "dilema do crescimento compensatório" (62).

É possível concluir, portanto, que além do período fetal e dos primeiros anos de vida, a transição da infância para a adolescência e vida adulta também são etapas que devem ser monitoradas em relação ao crescimento, a fim de garantir uma melhor saúde em idades posteriores (57).

# Revisão de literatura

Para a revisão de literatura que compõe este projeto, foram realizadas duas buscas sistemáticas nas bases de dados PUBMED, LILACS e WEB OF SCIENCE. Em ambas buscas, foi usado o termo "*Hemoglobin A, Glycosylated*" para o desfecho, de acordo com os termos de *Medical Subject Headings* (MeSH). Na estratégia de busca, o descritor do desfecho foi usado junto com diferentes descritores de variáveis independentes**.** Considerando que a HbA1c tem sido avaliada, principalmente, em estudos com população diabética, na sintaxe de busca foi especificada a exclusão do termo "Diabetes Melllitus". Não foram usados limites para idioma e data de publicação.

A primeira busca teve por objetivo conhecer a epidemiologia da HbAc1 em adolescentes e adultos sem diagnóstico de DM (distribuição efatores contemporâneos associados). Como descritores das variáveis independentes, foram utilizados os seguintes termos do MeSH: "Risk Factors", "Body Mass Index", "Waist Circumference", "Body Fat Distribution", "Ethnic Groups".

Painel 1. Termos de busca para artigos que avaliaram fatores contemporâneos associados com HbAc1

| |
|---|
| (((((("Hemoglobin A, Glycosylated"[Mesh]) AND ("Risk Factors"[Mesh] OR "Ethnic Groups"[Mesh] OR "Body Mass Index"[Mesh] OR "Waist Circumference"[Mesh] OR "Body Fat Distribution"[Mesh]) NOT ("Diabetes Mellitus"[Mesh]) |

Na segunda busca, buscou-se investigar os fatores precoces associados com a HbA1c, sobretudo fatores relacionados ao crescimento ao longo do ciclo vital. Os termos usados como descritores das exposições foram: “Birth Weight”; "Weight Gain"; "Growth and Development"; "Body Mass Index", "Fetal Growth Retardation"; "Nutritional Status", “Growth/physiology*” e "Child Development/physiology*". Destes, somente os dois últimos não são termos MeSH. Também foram incluídos nesta busca, os termos "Cohorts Studies" e "Longitudinal Studies".

Painel 2. Termos de busca para artigos que avaliaram fatores precoces associados com HbAc1

| |
|---|
| (((((((("Hemoglobin A, Glycosylated"[Mesh]) AND ("Birth Weight"[Mesh] OR "Weight Gain"[Mesh] OR "Nutritional Status" OR "Growth and Development"[Mesh] OR "Body Mass Index"[Mesh] OR "Fetal Growth Retardation"[Mesh] OR "Growth/physiology" OR "Child Development/physiology") AND ("Longitudinal Studies"[Mesh] OR "Cohort Studies"[Mesh]) NOT "Diabetes Mellitus" [Mesh]) |

Para a seleção dos estudos que compõem esta revisão foram utilizadas as seguintes estratégias:

- ✓ Busca nas bases de dados utilizando os descritores acima;
- ✓ Leitura minuciosa dos títulos, seleção destes e exclusão de duplicatas;
- ✓ Leitura dos resumos dos artigos julgados importantes a partir da leitura dos títulos;
- ✓ Identificação e leitura dos artigos relevantes na íntegra.

Nas seguintes seções, serão apresentados os resultados das duas buscas individualmente.

## Busca 1: Distribuição da hemoglobina glicada em população não diabética

O objetivo desta busca foi conhecer a distribuição da HbAc1 e fatores associados em populações comparáveis às Coortes de 1982 e 1993 de Pelotas. Portanto, não foram incluídos estudos que avaliaram crianças, população adulta acima de 40 anos e/ou idosa, ou populações específicas, incluindo por exemplo, obesos ou trabalhadores de algum tipo de indústria.

A Tabela 2 mostra os resultados da busca sobre distribuição da hemoglobina glicada em população não diabética.

**Tabela 2.** Resultado da revisão de literatura sobre epidemiologia da HbA1c, realizada nas bases Pubmed, Lilacs e Web of Science.

| Fonte | Registros encontrados | Títulos selecionados | Resumos selecionados | Artigos relevantes |
|---|---|---|---|---|
| Pubmed | 625 | 35 | 7 | 4 |
| Lilacs | 758 | 16 | 2 | 1 |
| Web of Science | 217 | 6 | 2 | 0 |
| Total | 1600 | 57 | 11 | 5 |

Nesta primeira revisão, dos 1600 registros recuperados, 57 foram selecionados pelo título e, destes, onze foram escolhidos após leitura do resumo. Finalmente cinco artigos foram selecionados como relevantes para este projeto após leitura na íntegra (Quadro 1).

Em todos os artigos, a HbA1c foi analisada como variável contínua, sendo que em um estudo foi avaliada, também, em quartis e decis (Nguyen et al). Dos seis estudos selecionados, três analisam os dados do NHANES III *(Third National Health and Nutrition Examination Survey)* e dois fazem parte de estudos de coorte, sendo um de base comunitária na cidade de Los Angeles, nos Estado Unidos, e outro em três comunidades no Japão.

Os aspectos mais relevantes dos estudos incluídos nesta busca encontram-se resumidos no Quadro 1.

**Quadro 1.** Estudos que avaliaram a distribuição de HbA1c e fatores associados em populações sem diagnóstico de diabetes mellitus.

| Autor e ano de publicação | Local | Amostra - Delineamento | N | Exposições | Idade mensuração do desfecho | Resultados | Ajustes |
|---|---|---|---|---|---|---|---|
| Kayaba et al, 1998 | Japão | *Jichi Medical School Cohort Study* (coorte prospectiva de base populacional) Comunidades de Yamato, Sakugi e Okawa. 1993 a 1995. | 2800 | Triglicerídeos, Colesterol total e HDL, Fibrinogênio, Lipoproteína A, Glicose em jejum, PA sistólica e diastólica, Tabagismo, Consumo de álcool. | 30 a 69 | Coeficiente de correlação de Pearson entre HbA1c e: - triglicerídeos: 0,18 - fibrinogênio: 0,19 - glicose em jejum: 0,73 Somente em mulheres: - IMC: 0,18 - colesterol total: 0,18 Em todos os indivíduos: p<0,001. Maiores médias em pessoas que consomem álcool e fumantes. | Idade. |
| Winkleby et al., 1999 | Estados Unidos | NHANES III. | 7686 | Etnia: brancos, americanos/mexicanos, negros. | 6 a 24 | Médias de HbA1c maiores para negros e mexicanos/americanos em relação aos brancos em todos os grupos de idade. | Idade, Escolaridade chefe de família. |

**Quadro 1.** Estudos que avaliaram a distribuição de HbA1c e fatores associados em populações sem diagnóstico de diabetes mellitus.

*(continuação)*

| Autor e ano de publicação | Local | Amostra – Delineamento | N | Exposições | Idade mensuração do desfecho | Resultados | Ajustes |
|---|---|---|---|---|---|---|---|
| Saaddine et al., 2002 | Estados Unidos | NHANES III. | 7968 | Idade, sexo, etnia, nível socioeconômico (NSE), história familiar de DM, sobrepeso e glicemia de jejum (2809 indivíduos maiores de 12 anos). | 5 a 24 | Diferenças pequenas entre categorias. Associação positiva entre HbA1c com homens, negros não hispânicos, mexicanos/americanos, indivíduos entre 10 e 14 anos, sobrepeso, com história familiar de DM, e glicemia em jejum maior que 126 mg/dl. Modelo 2: associação positiva com negros não hispânicos e mexicanos/americanos. | Modelo 1: Idade, sexo, escolaridade, familiar de DM, sobrepeso. Modelo 2: modelo 1 + glicemia de jejum. |
| Eldeirawi & Lipton, 2003 | Estados Unidos | NHANES III. | 4928 | Sexo, idade, IMC, IMC materno, NSE, etnia. | 4 a 17 | Maiores médias em homens *vs*. mulheres, e africanos/americanos e mexicanos/americanos comparados com brancos não hispânicos. Análises ajustadas: Associação positiva com idade e IMC. Associação negativa com NSE. | Sexo, etnia, idade. |

**Quadro 1.** Estudos que avaliaram a distribuição de HbA1c e fatores associados em populações sem diagnóstico de diabetes mellitus.

*(continuação)*

| Autor e ano de publicação | Local | Amostra – Delineamento | N | Exposições | Idade mensuração do desfecho | Resultados | Ajustes |
|---|---|---|---|---|---|---|---|
| Nguyen et al., 2008 | Los Angeles -Estados Unidos | Estudo prospectivo biracial (negro / branco) de base comunitária: Bogalusa Heart Study Acompanhamento do 2000/01. | 1203 | Idade, etnia, sexo Tabagismo, pressão arterial (PA), razão colesterol total/HDL, HOMA RI, proteína C reativa, adiponectina, história familiar de DCV e DM. | 24 a 43 | Preditores independentes de HbA1c: etnia negra, sexo feminino (β=0,16), razão colesterol total/HDL (β=0,10), CC (β=0,11)<br><br>Prevalência de excesso de HbA1c (decil mais alto) foi 1,6 vezes maior entre indivíduos com síndrome metabólica; 2,1 e 1,4 para aqueles com história familiar de DCV e DM, respectivamente | Modelo 1: idade, raça, sexo, tabagismo, PA, razão colesterol total/HDL, HOMA RI, proteína C reativa, adiponectina Modelo 2: modelo 1 + CC |

NHANES III: *Third National Health and Nutrition Examination Survey*; HbA1c: hemoglobina glicada fração A1c; IMC: índice de massa corporal; CC: circunferência da cintura; DM: diabetes mellitus; DCV: doença cardiovascular; HDL: colesterol HDL; PA: pressão arterial; HOMA RI: *homeostatic model assessment* para resistência à insulina; NSE: nível socioeconômico.

Kayaba et al., avaliaram os níveis de HbA1c e a correlação com outros fatores de risco cardiovascular em uma coorte de base populacional no Japão. Os participantes foram adultos entre 30 e 69 anos de idade e apresentaram médias de HbA1c de 5,61% e 5,49% em homens e mulheres, respectivamente. Observaram-se correlações positivas com triglicerídeos, fibrinogênio e glicemia de jejum, e correlações negativas com colesterol HDL em ambos os sexos. Pressão arterial, IMC e colesterol total apresentaram correlação positiva com os níveis de HbA1c somente em mulheres. Em todos os indivíduos, as correlações foram estatisticamente significativas ($p<0,05$), porém com coeficientes baixos ($r <0,20$), exceto para glicemia em jejum, que apresentou uma boa correlação com a HbA1c ($r= 0,73$)(63).

Os três estudos NHANES III utilizaram dados da primeira e segunda fases do inquérito, realizadas nos períodos de 1988 a 1991 e 1991 a 1994, respectivamente. Winklebey et al. analisaram a relação entre etnia e HbA1c. Os resultados apontaram para maiores médias de HbA1C em negros e mexicanos/americanos comparados aos de etnia branca(64). No estudo de Saaddine et al., foi avaliada a distribuição da HbA1c segundo características sociodemográficas e de saúde. Observou-se maiores médias de HbA1c no sexo masculino, em negros não hispânicos, em indivíduos com sobrepeso, com história familiar de DM e com intolerância à glicose (glicemia em jejum > 126 mg/dl), sendo que as diferenças foram pequenas. Ambos os estudos avaliaram indivíduos entre cinco e 24 anos de idade(65). Por último, Eldeirawi & Lipton estudaram os preditores da HbA1c em pessoas de quatro a 17 anos de idade com o objetivo de identificar crianças e adolescentes com risco aumento para o desenvolvimento de DM. A HbA1c encontrou-se associada positivamente com idade e IMC e, negativamente, com nível socioeconômico. Consistentemente com os outros estudos, as médias de HbA1c foram maiores na etnia de africano-americanos e mexicano-americanos em relação aos brancos não hispânicos(66).

Nguyen et al. avaliaram a distribuição de HbA1c e fatores independentes em adultos de 24 a 43 anos de idade. Raça negra, sexo feminino, razão colesterol total/ HDL elevada e o aumento da circunferência de cintura (CC) se mostraram como preditores independentes para maiores níveis séricos de HbA1c. A HbA1c foi analisada em decis, sendo que o decil mais alto esteve associado à presença de síndrome metabólica, história familiar de DCV e DM (67).

Em todos estes estudos relatados, as análises foram ajustadas. As variáveis de ajuste mais utilizadas foram sexo, idade, escolaridade, história familiar de DM ou de DCV e IMC. Também foram utilizadas consumo de álcool, tabagismo, nível de atividade física e glicemia em jejum.

## Busca 2: Determinantes precoces da hemoglobina glicada em qualquer etapa do ciclo vital

Os resultados desta busca são apresentados na Tabela 3.

**Tabela 3.** Resultado da revisão de literatura dos determinantes precoces da HbA1c, realizada nas bases de dados Pubmed, Lilacs e Web of Science.

| Fonte | Registros encontrados | Títulos selecionados | Resumos selecionados | Artigos relevantes |
|---|---|---|---|---|
| Pubmed | 153 | 16 | 8 | 4 |
| Lilacs | 19 | 2 | 1 | 1 |
| Web of Science | 13 | 0 | 0 | 0 |
| Total | 185 | 49 | 9 | 5 |

A maioria de estudos encontrados foram estudos clínicos ou com populações muito específicas. Os temas que mais frequentemente apareceram foram em relação à HbA1c e cirurgia bariátrica, ovários policísticos, hormônio do crescimento e diabetes gestacional, os quais foram excluídos.

Após exclusão dos títulos repetidos, foram lidos os resumos de nove artigos. A partir destes, cinco foram considerados relevantes.

Todos os estudos avaliaram como desfecho os níveis de HbA1c em qualquer etapa da vida (Quadro 2), sendo que dois foram realizados em países de média e baixa renda (Brasil e Jamaica) e três em países de alta renda (Polônia e Reino Unido). Dois artigos analisaram dados pertencentes à mesma amostra (Reino Unido). Nos cinco casos, os resultados de HbA1c foram apresentados em percentagem e não foram utilizados pontos de corte clínicos. Em um dos artigos do Reino Unido, os níveis de HbA1c também foram analisados de forma dicotômica, usando como ponto de corte 7%, porém estes resultados não serão apresentados nesta revisão, uma vez que, como mencionado anteriormente, esse valor indica presença de DM.

A seguir, serão resumidos os resultados da relação da HbA1c com fatores precoces, segundo os estudos revisados.

**Quadro 2.** Estudos que avaliaram a associação entre fatores precoces com níveis de HbA1c (%) em qualquer etapa do ciclo vital.

| Autor e ano de publicação | Local | Amostra – Delineamento | N | Exposições | Idade mensuração do desfecho | Resultados | Ajustes |
|---|---|---|---|---|---|---|---|
| Forrester et al. 1996 | West Indies, Jamaica | Coorte retrospectiva de 27 escolas do Hospital Universitário | 2337 | Peso ao nascer (PN), comprimento, perímetro cefálico e índice ponderal (IP) ao nascer<br>Pregas cutâneas (contemporâneas à medição do desfecho) | 6 a 10 | Aumento nos níveis de HbA1c conforme diminuição de comprimento ao nascer*.<br>HbA1c não esteve associada com PN, IP e perímetro cefálico ao nascer. | Idade, sexo e peso atual |
| Nazmi et al. 2007 | Pelotas, Brasil | Subamostra de meninos da Coorte de Nascimentos de 1982 de Pelotas | 197 | PN,<br>peso para idade gestacional | 18 | Relação inversa entre peso/idade gestacional (em quartis) e média de HbA1c<br>β= -0,08 (-0,15; -0,07)<br>Não houve associação com PN | Renda e escolaridade materna ao nascimento, IMC atual |
| Szostak-Węgierek et al. 2007 | Warsaw, Polônia. | Indivíduos nascidos em Warsaw entre 1974 e 1977, cujas mães tinham participado de um estudo prospectivo sobre fatores de risco de baixo peso ao nascer (BPN) | 498 | PN,<br>comprimento e IP ao nascer. | 24 a 29 | No sexo masculino, observou-se uma correlação negativa somente com IP<br>(r= – 0,165, p<0,05) | - |

* Dados completos para 475 crianças.

**Quadro 2.** Estudos que avaliaram a associação entre fatores precoces com níveis de HbA1c (%) em qualquer etapa do ciclo vital.

*(continuação)*

| Autor e ano de publicação | Local | Amostra – Delineamento | N | Exposições | Idade mensuração do desfecho | Resultados | Ajustes |
|---|---|---|---|---|---|---|---|
| Cooper et al. 2009 | Inglaterra, Escócia e Wales, Reino Unido | Coorte de nascimentos britânica de 1958. | 7855 | Idade gestacional (continua e categórica: <37, 37, 38, 39, 40, 41 e mais de 41 semanas). | 45 | Não houve associação | Peso/idade gestacional, sexo, IMC atual |
| Power, Thomas, 2011 | Inglaterra, Escócia e Wales, Reino Unido | Coorte de nascimentos britânica de 1958. | 7855 | Ganho de IMC em intervalos de tempo: 0 a 7, 7 a 11, 11 a 16, 16 a 22, 22 a 33, 33 a 45 anos. Duração/início de obesidade e sobrepeso (exemplo com obesidade: nunca obeso; obeso só aos 7, 11 ou 16; obeso aos 7, 11 ou 16 e aos 22 ou 33; obeso só aos 22 ou 33, obeso só aos 45, sempre obeso. | 45 | Em cada intervalo de tempo: associação positiva entre ganho de IMC e HbA1c (tendo em conta IMC ao início do intervalo).<br><br>Associação positiva com duração da obesidade. Indivíduos com obesidade somente aos 7, 11 ou 16 não mostraram maiores médias de HbA1c. Padrão similar de resultados com duração de sobrepeso e obesidade. Porém, perdeu significância após ajuste. Sem diferenças entre sexos. | Modelo com ganho de IMC: Sexo, história familiar de DM, tratamento atual DM, etnia, classe social precoce e contemporânea. Inicio de sobrepeso/ obesidade: ajustes anteriores: escolaridade, consumo álcool, fumo, menopausa, colesterol total e HDL, IMC e CC atuais. |

PN: peso ao nascer; PBN: baixo peso ao nascer; HbA1c: hemoglobina glicada; IP: índice ponderal; IMC: índice de massa corporal; CC: circunferência da cintura; DM: diabetes mellitus; HDL: colesterol HDL.

No estudo de Forrester et al., diversos fatores de risco cardiovasculares foram avaliados, em crianças com idade entre seis e dez anos, em relação ao crescimento fetal e alguns fatores contemporâneos. A amostra foi composta por crianças em idade escolar que tinham nascido no hospital universitário da capital da Jamaica. Como desfechos, foram examinados a pressão arterial, colesterol sanguíneo e hemoglobina glicada. Os dados do nascimento foram obtidos de registros hospitalares. As exposições contemporâneas mensuradas incluíram peso, estatura, pregas cutâneas, circunferência da cintura e do quadril. A média de HbA1c foi de 6,01%. Os resultados mostraram uma relação inversa, com tendência linear, entre comprimento ao nascer e níveis de HbA1c. Também foi observada relação inversa com prega tricipital aos 6-10 anos de idade. A HbA1c não esteve associada com PN, índice ponderal (IP) e perímetro cefálico ao nascer(68).

Em 2007, Nazmi et al. estudaram a relação entre crescimento intrauterino e HbA1c em uma subamostra de meninos de dezoito anos de idade pertencentes à Coorte de Nascimentos de 1982 de Pelotas, Brasil. As exposições avaliadas foram peso ao nascer (PN) e peso para idade gestacional. Este último foi examinado em quartis, para evitar problemas inerentes ao ponto de corte. A média de HbA1c na amostra foi de 5,22%. Foi encontrada uma associação inversa entre os níveis de HbA1c e a prevalência de baixo peso para idade gestacional(69).

Szostak-Węgierek et al. estudaram a associação entre fatores precoces e contemporâneos com diversos fatores de risco cardiovasculares (pressão arterial, perfil lipídico, resistência à insulina, fibrinogênio e glicose) mensurados em indivíduos adultos dos quais tinham informações do nascimento. As análises, estratificadas por sexo, foram realizadas por meio de correlações. PN e IP foram avaliados e somente foi encontrada associação linear inversa de HbA1c e IP em homens. Entre os fatores contemporâneos, foi encontrada uma relação direta de HbA1c com IMC, CC e razão cintura-quadril (RCQ) (correlações entre 0,17 e 0,19, $p<0,05$) (70).

Dois artigos utilizaram dados de uma coorte de nascimentos britânica para avaliar níveis séricos de HbAc1 aos 45 anos de idade. Por um lado, Cooper et al. estudaram a relação entre HbA1c e idade gestacional, sem encontrar associação(71). O segundo artigo com dados desta coorte teve como objetivos avaliar a existência de períodos críticos ou sensíveis de ganho de adiposidade para o metabolismo de glicose e os efeitos permanentes da obesidade na infância, com persistência ou não até a vida adulta. As principais conclusões foram, em primeiro lugar, que o ganho excessivo de IMC em qualquer etapa da vida estava relacionado com maiores

médias de HbA1c na vida adulta, sendo que o IMC atingido na idade adulta (contemporâneo ao desfecho) se mostrou como importante preditor dos níveis de HbA1c. As associações entre o início precoce de sobrepeso ou obesidade e os níveis de HbA1c na vida adulta foram, em grande parte, devido à maior adiposidade aos 45 anos. Em segundo lugar, indivíduos com sobrepeso na infância que apresentaram IMC normal na vida adulta não apresentaram maiores médias do desfecho, sugerindo que o efeito prejudicial do sobrepeso na infância pode ser melhorado com o ganho de IMC controlado na adolescência e vida adulta (72).

## Conclusões sobre a revisão de literatura

- ✓ Os estudos são controversos em relação às diferenças entre níveis de HbA1c conforme o sexo.
- ✓ Há evidências de que populações de etnia negra apresentam maiores níveis de HbA1c em relação às brancas.
- ✓ Em adultos, a HbA1c esteve associada positivamente com a razão colesterol total/ HDL, CC e síndrome metabólica.
- ✓ Apenas cinco estudos sobre determinantes precoces da HbA1c foram encontrados, o que justifica a realização das análises longitudinais aqui propostas.

## Modelo conceitual

Com base nas evidências anteriormente apresentadas, foi elaborado um modelo conceitual descrevendo os fatores precoces e contemporâneos relacionados à HbA1c em adolescentes e adultos (Figura 3).

O modelo apresenta a etnia no primeiro nível, uma vez que está bem documentado na literatura que esta variável é um determinante muito importante nos níveis de HbA1c nos seres humanos. Cada nível exerce influência sobre os níveis seguintes (mais próximos ao desfecho), em ordem hierárquica e temporal dos acontecimentos (73).

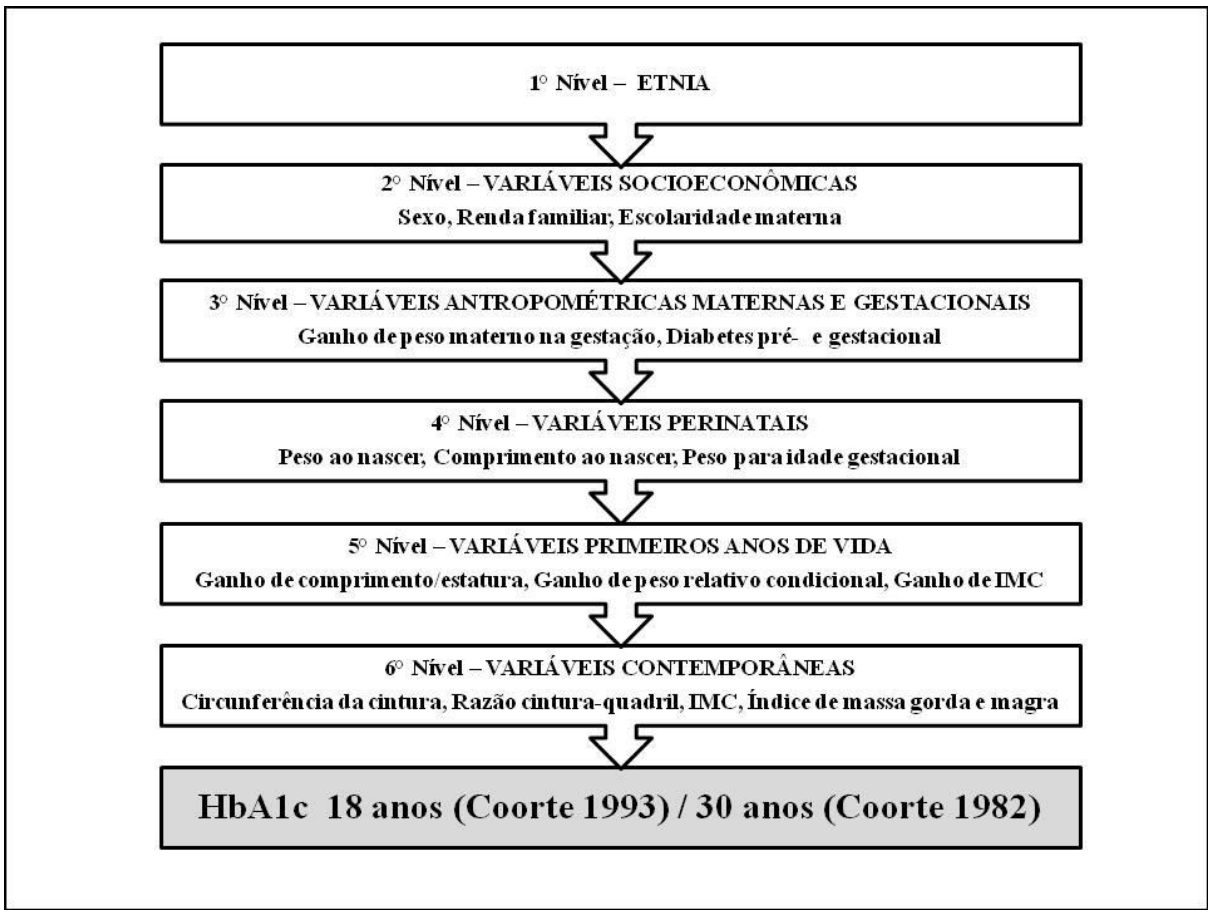


Figura 3. Modelo conceitual da associação entre hemoglobina glicada em adolescentes e adultos e variáveis precoces e contemporâneas.

# Justificativa

As DCV, consideradas um grave problema de saúde pública, comprometem as condições de saúde e qualidade de vida e são responsáveis por grande número de óbitos na população mundial (1). No Brasil, embora a incidência esteja diminuindo, as DCV continuam sendo a principal causa de morte, gerando altos custos referentes a internações hospitalares no sistema de saúde nacional (2). Estas tendências, encontradas em nível nacional e mundial, têm provocado grande interesse na pesquisa sobre este tema.

Diversas pesquisas têm sido realizadas com o intuito de conhecer os determinantes das DCV. Entre diversos fatores investigados, estudos clínicos e populacionais têm identificado a HbA1c como marcador de risco de DCV, tanto em indivíduos diabéticos como em não diabéticos (7, 40, 74). Por outro lado, estudos sobre a origem fetal das doenças crônicas ganharam especial atenção, mostrando que as doenças crônicas, assim como seus fatores de risco, são influenciadas por condições que atuam em etapas iniciais da vida e que têm repercussão em idades futuras (45). Estes estudos têm avaliado a relação entre fatores de risco para DCV ou DM com determinantes precoces, como peso ao nascer, estado nutricional nos primeiros anos de vida, ganho de peso e ganho de IMC (49). Entre estes fatores, os padrões de crescimento em diferentes etapas do ciclo vital são de especial relevância, já que demonstraram ter influências importantes no desenvolvimento de DCNT e outras condições na vida adulta (54, 75). O ganho de peso e ganho de IMC acelerados na infância e adolescência, por exemplo, se mostraram como fatores importantes no desenvolvimento de alterações cardíacas e metabólicas na vida adulta.

Assim, este estudo se justifica pela necessidade de melhor entendimento sobre a epidemiologia da HbA1c, um importante marcador de risco de DCV, assim como a relação deste desfecho com os padrões de crescimento ao longo do ciclo vital. Fatores de risco podem ser detectados em adolescentes e adultos jovens, muitos anos antes do início clínico das doenças (76-78), o que abre uma perspectiva importante para prevenir o desenvolvimento de doenças crônicas na vida adulta. Neste sentido, dados das Coortes de Nascimentos de Pelotas são de grande valor para investigar as relações expostas acima.

# Objetivos

## Objetivo geral

Este projeto de pesquisa tem como objetivo principal avaliar as associações entre os níveis de HbA1c de adolescentes e adultos com:

- ✓ Fatores genéticos, demográficos, socioeconômicos, comportamentais, estado nutricional e de composição corporal contemporâneos;
- ✓ Crescimento intra e extrauterino durante a infância e adolescência.

## Objetivos específicos

- ✓ Realizar revisão sistemática dos estudos que investigaram a relação entre BPN e ganho de peso acelerado com desfechos relacionados à alteração no metabolismo da glicose em qualquer etapa da vida.

- ✓ Descrever os níveis de HbA1c obtida de adolescentes com 18 anos (Coorte de Nascimentos de 1993) e de adultos com 30 anos (Coorte de Nascimentos de 1982) conforme variáveis contemporâneas, incluindo:
  - Ancestralidade genômica (possível apenas na Coorte de 1982);
  - Fatores demográficos e de posição socioeconômica (sexo, cor da pele, renda, índice de bens e escolaridade);
  - Fatores comportamentais (nível de atividade física, tabagismo, consumo de álcool);
  - Estado nutricional (IMC);
  - Composição corporal (índices de massa gorda e massa magra);
  - Indicadores de adiposidade abdominal (CC, RCQ).

- ✓ Avaliar a associação entre o nível de HbA1c de adolescentes com 18 anos (Coorte de Nascimentos de 1993) e as seguintes exposições:
  - Tamanho ao nascer (peso e comprimento);

- Peso para idade gestacional;
- Padrões de crescimento (ganho comprimento/estatura condicional, ganho de peso relativo condicional e ganho de IMC) durante infância e adolescência.

✓ Avaliar a associação entre fatores precoces e contemporâneos com a trajetória dos níveis séricos de HbA1c dos 18 aos 30 anos em uma subamostra de indivíduos do sexo masculino pertencentes à Coorte de Nascimentos de 1982.

# Hipóteses

1. As médias de HbA1c são maiores em meninos, indivíduos de cor da pele negra, de nível socioeconômico mais baixo, menor escolaridade, fumantes, com prática insuficiente de atividade física, obesos, com maiores índices de massa gorda e maiores CC e RCQ;
2. Existe associação inversa entre tamanho ao nascer (peso e comprimento) e HbA1c;
3. Existe associação inversa entre peso para idade gestacional e HbA1c;
4. Existe associação direta entre ganho de peso e de IMC acelerados durante infância e adolescência com a HbA1c;
5. Indivíduos que ganharam peso ou IMC rapidamente nos primeiros anos de vida apresentam maiores níveis de HbA1c do que indivíduos que não ganharam peso ou IMC rapidamente;
6. Fatores precoces e contemporâneos adversos (BPN, nível socioeconômico baixo, sobrepeso e obesidade abdominal) estão associados com aumento nos níveis séricos de HbA1c dos 18 para os 30 anos.

# Metodologia

## Delineamento

O presente estudo será longitudinal, prospectivo e utilizará dados das Coortes de Nascimentos de Pelotas de 1982 e 1993.

## Metodologia da Coorte de Nascimentos de 1982

A Coorte de Nascimentos de Pelotas do ano de 1982 incluiu todos os nascimentos hospitalares (99,2% do total de nascimentos na cidade) da área urbana do município de Pelotas-RS, entre o dia primeiro de janeiro até o dia 31 de dezembro. No respectivo ano, ocorreram 6011 nascimentos nas maternidades da cidade, sendo que, 5914 nascidos vivos fizeram parte do acompanhamento perinatal. O peso foi aferido (mas não o comprimento) e suas mães responderam a um questionário sobre aspectos socioeconômicos, demográficos e de saúde.

A partir do estudo inicial, foram realizados diversos acompanhamento na infância, adolescência e vida adulta, que possibilitaram diferentes estudos sobre a saúde destes indivíduos. Maiores detalhes dos acompanhamentos estão descritos em artigos metodológicos da Coorte (79, 80).

O Quadro 3 apresenta os acompanhamentos que serão utilizados no presente estudo.

**Quadro 3.** Acompanhamentos da Coorte de 1982 que serão utilizados neste estudo. Pelotas, RS.

| Ano | Idade | Estratégia amostral | Indivíduos entrevistados (N) | Perdas (%) |
|---|---|---|---|---|
| 1982 | Nascimento | Todos os nascimentos nas três maternidades | 5914 | - |
| 1983 | 11,3 meses | Todas as crianças nascidas de janeiro a abril | 1916 | 20,7 |
| 1984 | 19,4 meses | Todos os membros da coorte | 5914 | 12,8 |
| 1986 | 43,1 meses | Todos os membros da coorte | 5914 | 15,9 |
| 2000 | 18,2 anos | Todos os jovens do sexo masculino | 2250 | 21,1 |
| 2012 | 30 anos | Todos os membros da coorte | 5914 | 32,2 |

Fonte: Adaptada de Barros et al, 2008 (80).

***Subamostra do acompanhamento de 2000:*** No ano 2000, ocorreu o alistamento militar, obrigatório para todos os jovens do sexo masculino. Durante o exame médico militar, os jovens da coorte de 1982 foram identificados e entrevistados. Desta amostra, foi selecionada uma subamostra para realizar um estudo sobre função pulmonar. Para esta subamostra, foram selecionados todos os nascidos com baixo peso (n=118). Para cada um desses meninos nascidos com baixo peso, dois meninos com peso ao nascer apropriado (>2,500 g) foram selecionados aleatoriamente. Ao total foram escolhidos 345 meninos, para os quais se tem informação de hemoglobina glicada (69).

**Metodologia da Coorte de Nascimentos de 1993**

Todas as mães que tiveram partos hospitalares ocorridos na cidade de Pelotas-RS entre o dia primeiro de janeiro e 31 de dezembro de 1993 (n=6410) e que moravam na área urbana do

município foram visitadas por um integrante da equipe da pesquisa. Destas, 42 tiveram seus filhos em casa e foram levadas para o hospital posteriormente. Do total de nascidos, constituíram o estudo de base da coorte 5320 crianças. A taxa de perdas e recusas na etapa perinatal foi de 0,3%. Dentre as 5304 mães entrevistadas, 55 tiveram parto de feto morto. Portanto, a população final entrevistada em 1993 foi composta por 5249 filhos de mães residentes na zona urbana da cidade de Pelotas-RS que concordaram em participar do estudo. Foram coletadas informações antropométricas, demográficas, socioeconômicas e das condições de saúde das mães. Maiores detalhes metodológicos sobre os acompanhamentos da Coorte de Nascimentos de 1993 de Pelotas podem ser obtidos em publicação prévias (81-83).

O quadro abaixo apresenta os acompanhamentos realizados a serem utilizados no presente estudo, com os respectivos processos de amostragem, números de crianças elegíveis e taxas de acompanhamento.

**Quadro 4.** Acompanhamentos da Coorte de 1993 que serão utilizados neste estudo. Pelotas, RS.

| Ano | Idade | Estratégia amostral | Indivíduos elegíveis (N) | Perdas (%) * |
|---|---|---|---|---|
| **1993** | Nascimentos | Todos os nascimentos de 1993 na cidade de Pelotas | 5265 | 0,3 |
| **1993-4** | 1 mês | Amostragem sistemática de 13% da coorte inicial | 655 | 0,9 |
| **1993-4** | 3 meses | Idem ao anterior | 655 | 1,7 |
| **1993-4** | 6 meses | Todas as crianças nascidas com baixo peso (<2.500 g) e 20% dos restantes membros da coorte (inclusive aqueles acompanhados no primeiro e terceiro mês de vida) | 1460 | 3,2 |
| **1994-5** | 12 meses | Idem ao anterior | 1460 | 6,6 |
| **1997-8** | 4 anos | Idem ao anterior | 1460 | 12,8 |
| **2004-5** | 10-11 anos | Todos os membros da coorte | 5249 | 12,5 |
| **2008-9** | 14-15 anos | Todos os membros da coorte | 5249 | 14,3 |
| **2011-2** | 18-19 anos | Todos os membros da coorte | 5249 | 18,7 |

* Dados conhecidos de mortes foram considerados no cálculo

Fonte: Adaptada de Victora et al, 2006 e Gonçalves et al, 2014 (82, 83) .

## População em estudo

Coorte de Nascimentos de 1982: a população em estudo será composta por aqueles que fizeram parte dos acompanhamentos nos anos de 1982, 1983, 1983, 1986, 2000 e 2012.

Coorte de Nascimentos de 1993: a população em estudo será composta por aqueles integrantes que fizeram parte dos acompanhamentos nos anos de 1993, 1994, 1997, 2004, 2008 e 2011.

## Critérios de elegibilidade

### Critérios de inclusão

- Nascido em hospitais de Pelotas entre 1º de janeiro e 31 de dezembro de 1982 ou 1993;
- Família moradora da zona urbana no município de Pelotas;
- Selecionado para acompanhamento nas visitas que estudaram subamostras.

### Critérios de exclusão

- Ter nascido em casa e não ter sido levado ao hospital logo após o nascimento.
- Crianças que, no momento do nascimento, a família não residia na zona urbana do município de Pelotas.
- Grávidas ou possíveis grávidas na ocasião da entrevista dos acompanhamentos do 18 ou 30 anos;
- Apresentar diagnóstico de diabetes ou hemoglobina glicada ≥6,5% no momento de aferição do desfecho em estudo.

## Variável dependente

HbA1c em percentagem (g HbA1c/ g hemoglobina). Este desfecho será analisado como variável contínua.

**Dosagem de hemoglobina glicada**

A dosagem da HbA1c foi realizada através do programa Hemoglobina A1c VARIANT$^{TM}$ II, o qual é projetado para a determinação de hemoglobina A1c em sangue humano total, utilizando a cromatrografia líquida de alta performance (HPLC) associado à cromatrografia de troca iônica.

Na subamostra do ano 2000 da Coorte de 1982, a dosagem foi realizada utilizando o teste Abbott IMx Ion Capture Assay (Abbott Park, IL, USA).

## Variáveis independentes

Todas as variáveis que serão utilizadas para este estudo já estão coletadas e serão obtidas dos bancos de dados das Coortes de 1982 e 1993. As principais exposições de interesse são apresentadas no Quadro 5.

**Quadro 5.** Principais exposições a serem estudadas, segundo ano de coleta.

| Variável | Ano de coleta | | Descrição | Tipo |
|---|---|---|---|---|
| | Coorte 1982 | Coorte 1993 | | |
| Tamanho ao nascer | 1982 | 1993 | Peso ao nascer (g) | Contínua |
| | | | Baixo peso ao nascer (<2.500 g) | Dicotômica |
| | | | Comprimento ao nascer (cm) | Contínua |
| | | | Idade gestacional (IG) (semanas) | Contínua |
| | | | Peso para idade gestacional (escore Z) | Contínua e categórica (quartis) |
| Crescimento extrauterino* | - | 1993<br>1994<br>1997<br>2004 | Altura/comprimento condicional<br>Peso relativo condicional<br>IMC | Contínua |

| | | | | |
|---|---|---|---|---|
| | | 2008<br>2011/12 | | |

**Quadro 5.** Principais exposições a serem estudadas, segundo ano de coleta.

*(continuação)*

| Variável | Ano de coleta | | Descrição | Tipo |
|---|---|---|---|---|
| | Coorte 1982 | Coorte 1993 | | |
| Demográfico | 2012 | 2011 | Cor da pele autorreferida | Categórica |
| Nível socioeconômico | 2012 | 2011 | Renda (salários mínimos) | Contínua e categórica |
| | | | Índice de bens | Contínua e categórica |
| | | | Escolaridade (anos completos de estudo) | Contínua e categórica |
| Adiposidade central | 2012 | 2011 | CC e RCQ | Contínuas |
| Comportamentais | 2012 | 2011 | Atividade física | Contínua e dicotômica (≥150 min/sem) |
| | | | Tabagismo | Dicotômica |
| | | | Consumo de álcool | Dicotômica |
| Estado nutricional | 2012 | 2011 | IMC | Contínua |
| | | | Sobrepeso (IMC>25 kg/m$^2$) | Dicotômica |
| Composição corporal | 2012 | 2011 | Índices de massa gorda e magra | Contínua |
| Ancestralidade genômica | 2012 | - | Proporção de ancestralidade africana, europeia ou nativo- | Categórica |

| | | | | |
|---|---|---|---|---|
| | | | americana | |

*Será avaliado nos seguintes períodos: 0-1, 1-3, 3-6, 6-12 meses, 1-4, 4-11, 11-15, 15-18 anos; na Coorte de Nascimentos de 1993

No Quadro 6, são apresentadas as variáveis que serão utilizadas como possíveis fatores de confusão e o ano de acompanhamento correspondente à coleta.

**Quadro 6.** Possíveis fatores de confusão e mediadores a serem estudados, segundo ano de coleta.

| Ano de coleta | | Variável | Tipo |
|---|---|---|---|
| Coorte 1982 | Coorte 1993 | | |
| 1982 | 1993 | Sexo | Dicotômica |
| 1982 | 1993 | Renda familiar (salários mínimos) | Contínua<br>Categórica em quintis |
| 1982 | 1993 | Escolaridade materna (anos completos de estudo) | Discreta |
| 1982 | 1993 | Ganho de peso na gestação (kg) | Contínua |
| 1982 | 1993 | Diabetes pré-gestacional | Dicotômica |
| 2012 | 2011 | IMC ($kg/m^2$) | Contínua |

## Cálculo de poder amostral

Uma vez que as variáveis a serem utilizadas para o presente estudo foram coletadas previamente, não será realizado o procedimento normal para cálculo de amostra. Ao invés disso,

será realizado o cálculo do poder que seria possível detectar considerando diferenças de 0,5; 0,10 e 0,15; considerando um erro alfa de 5% e diferentes valores de desvios padrão. O cálculo será realizado para algumas das variáveis de exposição, sendo que o tamanho da amostra de cada Coorte está apresentado na Tabela 4.

Os cálculos de poder para as Coortes de 1993 e 1982 serão apresentados nas Tabelas 5 e 6, respectivamente.

Tabela 4. Tamanho de amostra de expostos e não expostos para cada coorte de nascimento

| Variáveis | Tamanho de amostra | |
|---|---|---|
| | Coorte 1982 | Coorte 1993 |
| Baixo peso ao nascer | | |
| Sim | 510 | 533 |
| Não | 4738 | 5375 |
| Déficit de altura para idade no segundo ano | | |
| Sim | 182 | 687 |
| Não | 1179 | 4242 |

Tabela 5. Cálculo de poder para detectar diferenças percentuais em relação ao desvio-padrão de HbA1c, considerando baixo peso ao nascer e déficit de altura para idade nos dois anos de vida na Coorte de 1982

| Mínimas diferenças detectáveis com alfa=5% | Poder (%) | | |
|---|---|---|---|
| | DP = 0,5 | DP = 1,0 | DP = 1,5 |
| Baixo peso ao nascer | | | |

| | | | |
|---|---|---|---|
| 0,05 | 57 | 19 | 11 |
| 0,10 | 99 | 57 | 30 |
| 0,15 | 100 | 90 | 57 |
| Déficit de altura para idade no primeiro ano de vida | | | |
| 0,05 | 24 | 9 | 7 |
| 0,10 | 71 | 24 | 13 |
| 0,15 | 96 | 47 | 24 |

DP: desvio-padrão

Tabela 6. Cálculo de poder para detectar diferenças percentuais em relação ao desvio-padrão de HbA1c, considerando baixo peso ao nascer e déficit de altura para idade no segundo ano de vida na Coorte de 1993

| Mínimas diferenças detectáveis com alfa=5% | Poder (%) | | |
|---|---|---|---|
| | DP = 0,5 | DP = 1,0 | DP = 1,5 |
| Baixo peso ao nascer | | | |
| 0,05 | 60 | 19 | 11 |
| 0,10 | 99 | 60 | 31 |
| 0,15 | 100 | 91 | 60 |
| Déficit de altura para idade no primeiro ano de vida | | | |
| 0,05 | 68 | 23 | 13 |
| 0,10 | 100 | 68 | 37 |
| 0,15 | 100 | 95 | 68 |

DP: desvio-padrão

É possível estimar diferenças iguais ou superiores a 0,10, sendo o DP= 0,5, com poder de 100%. O poder vai diminuindo à medida em que aumenta o DP.

## Logística

### Trabalho de campo

A seguir, será descrita a logística da coleta de sangue no último acompanhamento das Coortes de 1993 (18 anos) e 1982 (30 anos).

A coleta de sangue foi realizada na "Clínica", situada no Centro de Pesquisas Epidemiológicas Dr. Amilcar Gigante, e nos domicílios. Mulheres grávidas ou possíveis grávidas foram excluídas da coleta.

Nos acompanhamentos de ambas as Coortes, a equipe de trabalho foi treinada pela pesquisadora e bioquímica Isabel Oliveira e pela bióloga Helena Thurow.

O sangue e materiais de consumo (ponteiras, tubos, luvas, agulhas, entre outros) provenientes da coleta e do processamento eram autoclavados antes do descarte (calor úmido: 15minutos, 120°C). Todo lixo contaminado era armazenado em sacos brancos leitosos (lixo hospitalar) e o recolhimento desse lixo era realizado uma vez por semana, por uma empresa especializada, contratada pela Universidade, via Coordenadoria de Qualidade Ambiental, a qual era responsável pelo descarte e pela incineração.

Maiores detalhes da logística dos campos, podem ser acessados nos relatórios de trabalho de campo correspondentes.

### Revisão sistemática

A revisão será realizada por meio de uma busca sistemática da literatura nas bases de dados PubMed, Lilacs e Web of Science. Os termos de indexação e palavras-chave utilizadas serão identificados através dos artigos que foram encontrados na busca sistemática da realização deste projeto. O Painel 3 apresenta um exemplo dos termos de indexação mais frequentemente

utilizados. Esta seleção de termos será completada com a seleção de artigos derivada da revisão das referências das publicações selecionadas.

Painel 3. Exemplo para a seleção da chave de busca

| Author | Year | Birth Weight* | Birth Weight/ physiology | Body Weights and Measures | Infant, Small for Gestational Age | Gestational Age* | Growth/ physiology* | Weigth Gain | Newborn | Infancy | Child | Adolescent | Adult | Blood Glucose/ metabolism* | Blood Glucose/physiology | Glucose Tolerance Test | Glucose Intolerance/ epidemiology* | Glucose Intolerance /etiology* | Insulin Resistance | Diabetes Mellitus, Type 2/etiology* | Homeostasis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Norris | 2010 | x | | | x | | | | | | x | x | x | x | | | x | | | x | x |
| Fabricius-Bj | 2011 | x | x | | | | | | | | | | | x | | | | | | | |
| Adair | 2013 | x | x | | | | | | | | | | x | | x | | | | | x | |
| Thomas | 2012 | x | | x | | | | | | | | | x | | | | | | | | |
| Eriksson | 2011 | x | | | | | | | | | | | | | | | | | | | |
| Krishnaveni | 2010 | x | | | | | | | | | | | | | | | | | | | |
| Law | 1995 | x | | | | | | | | | | | x | x | | | | | | | |
| Yajnik | 1995 | x | | | | x | | | | | x | | x | x | | | | | | | |

Chave de busca:

((((("Adult"[MeSH Terms] OR "Infant"[MeSH Terms] OR "Infant, Newborn"[MeSH Terms] OR "child, Preschool"))) AND (("Birth Weight"[MeSH Terms] OR "Infant, Low Birth Weight"[MeSH Terms] OR "Weight Gain"[MeSH Terms]))) AND (("Blood Glucose"[MeSH Terms] OR "Diabetes Mellitus, Type 2"[MeSH Terms] OR "Glucose Intolerance"[MeSH Terms] OR "Glucose Tolerance test"[MeSH Terms] OR "Insulin Resistance"[MeSH Terms]))

Será criado um protocolo para a seleção dos artigos, com definição dos critérios de inclusão e exclusão, bem como ferramentas de avaliação da qualidade dos artigos. A seleção será feita por dois pesquisadores independentes.

**Controle de qualidade**

Para o controle de qualidade, foram realizados diversos procedimentos nos acompanhamentos das Coortes, entre os quais estão:

- Treinamento e aplicação de questionários padronizados;
- Treinamento de medidas antropométricas e biológicas;
- Calibração periódica dos equipamentos;
- Reuniões frequentes para discussão metodológica; presença constante da coordenadora e de dois supervisores durante os exames;

- Controle de qualidade das entrevistas de 10% da amostra, através da realização de ligações;
- Checagem de consistência dos dados.

## Análises dos dados

Devido à utilização de diversos acompanhamentos, inicialmente será apresentada, para cada coorte, uma tabela comparativa dos indivíduos acompanhados e dos não acompanhados em relação a algumas variáveis de interesse para o presente estudo.

Logo, será realizada uma análise exploratória da distribuição do desfecho através de inspeção visual de histogramas e medidas de simetria e curtose. O mesmo será avaliado de forma contínua, com algum tipo de transformação, se necessário, e como variável categórica por meio do uso de quintis ou decis. Os indivíduos com valores acima de 6,5% de HbA1c serão excluídos da análise, por ser este ponto de corte indicativo de diagnóstico de DM.

A descrição de variáveis contínuas será realizada através de medidas de tendências central e dispersão. As variáveis categóricas serão descritas por meio de frequências absolutas e relativas.

Para verificar a relação de cada uma das variáveis independentes com o desfecho, serão realizados testes paramétricos ou não paramétricos conforme a natureza da variável. Logo após, análises ajustadas serão realizadas para controle de possíveis fatores de confusão e modificadores de efeito, os quais serão incorporados em modelos de regressão. Será testada interação com sexo para decidir se a análise será estratificada por esta variável.

No artigo 3, para avaliar o efeito do crescimento sobre os níveis de HbA1c na Coorte de 1993, serão utilizadas medidas de crescimento condicional (peso e altura). Este tipo de variável é gerada a partir dos resíduos padronizados derivados de modelos regressão, onde o desfecho é o tamanho em determinada idade e as exposições são as medidas em idades anteriores. Seu uso é apropriado em estudos longitudinais, nos quais os indivíduos são acompanhados periodicamente, portanto suas medidas são altamente correlacionadas (84). Os resultados, apresentados em desvio padrão (DP), indicam se o crescimento durante um período de tempo é mais rápido ou mais lento de acordo com o esperado pelo crescimento médio dos indivíduos pertencentes à Coorte e pelas próprias medidas do indivíduo em idades anteriores (85). Pretende-se examinar no mesmo artigo

os efeitos do ganho de peso independentemente da altura. Sendo assim, serão utilizadas as variáveis de peso relativo ao crescimento linear e crescimento linear condicionais propostas por Adair et al. representando uma associação independente específica para cada idade. A altura condicional é o comprimento ou altura atual do indivíduo tendo em conta as medidas de comprimento ou altura e peso prévias (mas não o peso atual). O peso relativo condicional é o peso atual tendo em conta a altura atual e todas as medidas de peso e altura anteriores (75). A velocidade de ganho de IMC será analisada como variação média por período de tempo.

Estas análises seguirão o modelo que estabelece níveis hierárquicos de determinação apresentado na Figura 3. Conforme sugere a revisão da literatura, os níveis de HbA1c são maiores nos indivíduos de cor da pele negra em comparação à branca. Sendo assim, as análises serão estratificadas por cor da pele.

Na Coorte de 1993, a estratégia de amostragem dos acompanhamentos de 6, 12 e 48 meses sobrerrepresentou os participantes com baixo peso, exigindo análises ponderadas quando se utilizam dados desses acompanhamentos.

Todas as análises serão conduzidas no programa estatístico Stata 12.0 (StataCorp, Texas, USA).

# Aspectos éticos

O presente projeto será submetido ao Comitê de Ética em Pesquisa da Faculdade de Medicina da Universidade Federal de Pelotas.

Os acompanhamentos das Coortes que farão parte do presente estudo foram aprovados pelo Comitê de Ética da Faculdade de Medicina da Universidade Federal de Pelotas. Em todos os acompanhamentos das Coortes, foram assinados Termo de Consentimento Livre e Esclarecido por escrito pelos pais das crianças ou adolescentes e adultos entrevistados. Foi garantida a confidencialidade dos dados, a participação voluntária e a possibilidade de abandonar o estudo a qualquer momento, sem necessidade de justificativa.

# Cronograma

As atividades seguirão o cronograma apresentado a seguir, tendo duração prevista de 45 meses.

Quadro 7. Cronograma de atividades

| Atividade/Trimestres | 2013 | | | | 2014 | | | | 2015 | | | | 2016 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Elaboração do projeto | | | | | | | | | | | | | | | | |
| Revisão de literatura | | | | | | | | | | | | | | | | |
| Trabalho de campo* | | | | | | | | | | | | | | | | |
| Estágio no exterior | | | | | | | | | | | | | | | | |
| Análises da base de dados | | | | | | | | | | | | | | | | |
| Redação dos artigos | | | | | | | | | | | | | | | | |
| Entrega/defesa da tese | | | | | | | | | | | | | | | | |

*Uma vez que os dados já estão coletados, o trabalho de campo está sendo feito no acompanhamento pré-natal da Coorte de 2015.

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Para a criação da Coorte de 1993, os pesquisadores contaram com o financiamento da Comunidade Econômica Europeia. No entanto, para o desenvolvimento do estudo até os dias de hoje, diversas instituições têm contribuído, tais como: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes); *Wellcome Trust;* Programa Nacional para Centros de Excelência; Conselho Nacional de Pesquisa; Ministério da Saúde do Brasil; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS).

Além disso, a autora deste projeto é bolsista da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

# Divulgação dos resultados

Os resultados encontrados no estudo serão divulgados em diferentes veículos de comunicação. Os artigos científicos serão publicados em periódicos nacionais e/ou internacionais. Também serão divulgados na imprensa local, através de nota no jornal local e no site do Programa de Pós-graduação em Epidemiologia.

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# ALTERAÇÕES DO PROJETO DE PESQUISA

Nesta seção, são detalhadas as mudanças realizadas em 3 dos 4 propostos no projeto original ao longo do desenvolvimento da pesquisa.

**Artigo 1**

- Revisão sistemática: Padrões de crescimento e metabolismo da glicose

Este artigo previa uma revisão sistemática da literatura objetivando investigar a associação entre ganho de peso, ganho de índice de massa corporal e/ou ganho de estatura acelerados na infância e/ou adolescência e marcadores de hiperglicemia (concentração plasmática de glicose em jejum, concentração plasmática de glicose depois do teste oral de tolerância à glicose, níveis de HbA1c). Nas primeiras etapas da revisão, evidenciamos que vários dos estudos sobre metabolismo glicêmico também incluíam o índice HOMA-RI (modelo homeostático para avaliação da resistência à insulina) entre os desfechos. Por este motivo, decidimos incluir este indicador na nossa revisão. Tal mudança aumentou o espectro de desfechos o que permitiu explorar o efeito dos padrões de crescimento sobre alterações metabólicas de uma forma mais completa.

**Artigo 3**

- Crescimento e hemoglobina glicada em adolescentes: Coorte de Nascimentos de 1993 de Pelotas

No artigo inicialmente proposto no projeto de qualificação, planejava-se estudar como único desfecho, a concentração de HbA1c em adolescentes de 18 anos na Coorte de Nascimentos de 1993.

A utilização apenas deste desfecho trazia importante limitação à proposta, sobretudo em termos de publicação. Dado que a Coorte de 1993 tem informação disponível sobre diversos marcadores de risco cardiovascular, foi realizada a inclusão dos seguintes desfechos: concentração de glicemia ao acaso, proteína C-reativa, perfil lipídico (colesterol total, HDL colesterol, LDL colesterol e triglicerídeos), pressão arterial sistólica e diastólica, IMC e circunferência da cintura. Desta forma, ampliamos extensamente o escopo do artigo reduzindo a possibilidade de viés de publicação.

**Artigo 4**

- Trajetória de hemoglobina glicada dos 18 aos 30 anos: importância de fatores precoces e contemporâneos.

Este artigo, sugerido pelos dois membros da banca examinadora, foi incluído ao projeto, após a defesa do mesmo, em setembro de 2014. A Coorte de Nascimentos de 1982 de Pelotas avaliou os níveis de HbA1c em dois momentos: no ano 2000, quando uma subamostra de indivíduos do sexo masculino foi examinada no quartel do exército (participantes com18 anos de idade), e no ano 2012, no acompanhamento dos 30 anos. Sendo assim, o artigo tinha por objetivo avaliar a trajetória de HbA1c dos 18 aos 30 anos e fatores associados na subamostra de homens pertencentes à Coorte de Nascimentos de 1982 de Pelotas.

No entanto, a realização do artigo não foi possível devido a diferenças nos métodos de análises da HbA1c. No subestudo dos 18 anos os níveis de HbA1c foram avaliados utilizando o sistema Aboot IMx Ion Capture Assay (Abbot Park, IL, USA), um método baseado em afinidade de boronato. No acompanhamento dos 30 anos, o sistema escolhido foi o VARIANT$^{TM}$ II testing system (Bio-Rad Laboratories Inc, Hercules, CA), que utiliza o método de cromatografía líquida de alta eficiência de intercambio iónico.

Foram conduzidas análises exploratórias para avaliar a concordância de ambos os métodos, sendo observado que os valores de HbA1c variaram muito entre um e outro método, impossibilitando a realização do artigo. A seguir são apresentados resultados da comparação entre dados de HbA1c dos 18 e 30 anos.

Tabela 1. Descrição da variável HbA1c correspondente aos acompanhamentos dos 18 anos e 30 anos na subamostra de homens da Coorte de Nascimentos de 1982 - Pelotas/RS (N=187)

| HbA1c | Média (dp) | Mínimo | Máximo |
|---|---|---|---|
| 18 anos | 6.03(0.83) | 4.16 | 9.29 |
| 30 anos | 5.09(0.42) | 4 | 6.5 |

dp: desvio padrão

Concordância entre HbA1c correspondente aos acompanhamentos dos 18 anos e 30 anos na subamostra de homens da Coorte de Nascimentos de 1982 - Pelotas/RS (N=187)

Coeficiente de concordância de Lin: 0.015

Coeficiente de correlação de Pearson: 0.038

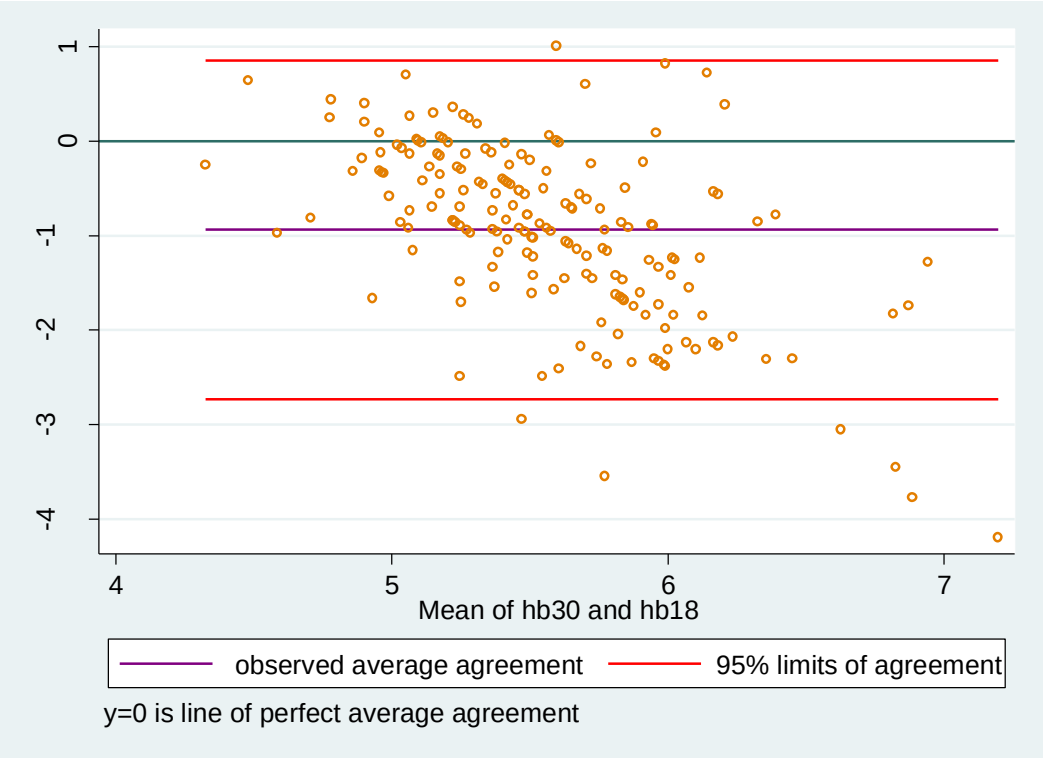


Gráfico 1. Gráfico Bland-Altman dos valores de HbA1c correspondentes aos acompanhamentos dos 18 anos e 30 anos na subamostra de homens da Coorte de Nascimentos de 1982 - Pelotas/RS (N=187)

# TRABALHO DE CAMPO

Como parte da formação e por exigência regimental do Programa de Pós-graduação em Epidemiologia (PPGE), a doutoranda participou da coleta de dados de uma das coortes de nascimentos de Pelotas.

Na ocasião do ingresso da doutoranda no programa (2013), a coleta dos dados utilizados no desenvolvimento da tese (oriundos do acompanhamento de 2011/12 e acompanhamento de 2012 das coortes de nascimentos de Pelotas de 1993 e 1982, respectivamente) já havia ocorrido. Por esse motivo, a experiência de campo da doutoranda deu-se na coorte de nascimentos de Pelotas de 2015, especificamente no acompanhamento pré-natal.

Do período de outubro de 2013 a fevereiro de 2014, a doutoranda atuou em diversas atividades relativas ao planejamento do trabalho de campo da referida coorte, como elaboração do questionário, planejamento da logística de recrutamento das gestantes, seleção e treinamento de entrevistadoras e visita às unidades de recrutamento. No período de março a setembro de 2014, a doutoranda atuou como supervisora de campo. O relatório de trabalho de campo do acompanhamento pré-natal será fornecido à banca via email.

No Apêndice A da tese, constam informações sobre a coleta sanguínea e dosagem da HbA1c nos acompanhamentos dos 30 e 18 anos das coortes de 1982 e 1993, respectivamente.

Os relatórios de trabalho de campo dos diferentes acompanhamentos da coorte de 1993, utilizados nesta tese, serão fornecidos via email através de um *link* na internet para a banca examinadora.

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# ARTIGOS

## Artigo 1 - Growth patterns during infancy, childhood and adolescence and glucose and insulin metabolism in adulthood: a systematic review

Foi submetido e está em revisão na revista Preventive Medicine

As normas de publicação da revista podem ser acessadas através do seguinte *link*: https://www.elsevier.com/journals/preventive-medicine/0091-7435/guide-for-authors

**Growth patterns during infancy, childhood and adolescence and glucose and insulin metabolism in adulthood: a systematic review**

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**Keywords:** growth; accelerated growth; blood glucose levels; type 2 diabetes; glucose intolerance; hyperglycaemia; insulin resistance; systematic review; qualitative analyses

**Manuscript word count:** 3289

**Abstract word count:** 246

**Abstract**

We systematically reviewed literature assessing the relationship between growth (change in weight, height or BMI) during infancy, childhood and adolescence and indicators of glucose and insulin metabolism in adulthood (fasting glycaemia, 2 hours glycaemia after an Oral Glucose Tolerance Test, hyperglycaemia, HOMA-IR). Electronic searches were carried out in Medline, Web of Science, SciELO and Lilacs (June 2016). Eligible studies were examined by two independent reviewers for relevance and methodological quality was rated based on the Newcastle-Ottawa Scale. Qualitative analysis was used to synthesize the results. We identified 4,876 records of which 23 were selected for full-text reading and 11 were relevant to the purpose of the review. All of them were cohort studies and considered as having high methodological quality. Some inconsistencies in growth definitions and age intervals were identified. Weight or BMI gain during the first two years of life were not associated with fasting glucose levels and hyperglycaemia, however, some evidence of a negative association with glucose concentrations after an oral glucose tolerance test was found. Gaining excessive weight/BMI during childhood and adolescence was found to be associated with all outcomes. Linear growth (change in length/height), examined only by two studies, appeared to have no effect on any of the outcomes assessed. The most consistent evidence was documented for rapid weight or BMI gain from childhood onwards and a worse glucose and insulin metabolism profile at adult age. This finding highlights the prevention of gaining excessive weight after the first two years of life.

## Introduction

Metabolic disturbances such as glucose intolerance and insulin resistance are associated with development of type 2 diabetes and cardiovascular diseases (1). According to the World Health Organization (WHO), hyperglycaemia (high blood glucose levels) is one of the five leading worldwide health risk factors for disability-adjusted life years (DALYs) and mortality due to cardiovascular deaths (2). Rates of type 2 diabetes will increase about 50% by 2040, causing enormous impact in human and economic costs (3). Therefore, there is an increased interest in the public health community to understand the mechanisms that explain the glucose and insulin disturbances in adulthood.

Evidence suggests that exposures occurring during early life, even during prenatal period, may have an important role in the risk of developing metabolic diseases (4). A systematic review addressing the relationship between birth weight and both later glucose and insulin metabolism showed that individuals who were light at birth had higher mean values of fasting and postprandial glucose, and greater risk of diabetes type 2 and insulin resistance later in life (5). A review conducted by Victora et al. concluded that intrauterine growth restriction or undernourishment in the first 2 years of life, followed by accelerated weight gain, contribute to development of insulin resistance and type 2 diabetes. The authors also reported inverse associations of fasting glucose levels with birth weight, weight-for-age, and body-mass-index-for-age at 2 years, after adjustment for adult BMI and height (6) . The fact that the associations between early life size and adult outcomes become apparent or stronger only when adult size is included in the model suggests that postnatal growth (i.e.: gaining excessive weight) is involved in these mechanisms (7) , which is relevant for public health interventions aiming to prevent chronic diseases. Furthermore, a review undertaken by Fisher et al. observed inconclusive evidence from three studies concerning infant size and non-insulin dependent diabetes in adult and elderly population (8).

However, to date, there are no comprehensive systematic reviews on the association between growth through infancy, childhood and adolescence and both glucose and insulin metabolism among adults. Thus, we aimed to systematically review published studies documenting the association between growth (change in weight, height or BMI) during infancy, childhood and adolescence and various indicators of glucose and insulin metabolism in

adulthood. With this review, we intended to contribute to the debate on critical periods of life for health interventions that aim prevention of type 2 diabetes and cardiovascular diseases.

## Methods

### Protocol and registration

This systematic review followed the guidance of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (9) and its protocol was registered in PROSPERO (International prospective register of systematic reviews) under the registration number CRD42015017188.

### Eligibility criteria

We carried out a systematic review of published literature looking for studies which reported the association between growth during infancy, childhood and adolescence (change in height, weight or BMI over time) and glucose and insulin metabolism in adulthood. We considered original articles that assessed at least two measurements of size (height, weight or BMI) collected at different times up to 18 years of age. The outcomes included were blood glucose concentrations measured as fasting glucose or two-hour concentration after glucose load, glycated hemoglobin, impaired glucose tolerance, impaired fasting glycaemia, type 2 diabetes and insulin resistance. We included insulin resistance measured by homeostatic model assessment (HOMA) as it is the most assessed indicator of insulin metabolism in population studies.

Studies based on population samples with specific health conditions such as low birth weight, small for gestational age or twin studies were not considered. We also excluded reviews, ecological studies, interventions and non-human studies. No limit was applied in relation to year of publication or language.

### Information sources and search strategy

We searched Medline (since 1946), Web of Science (since 1900), SciELO and Lilacs databases in June 20, 2016. We used the following search terms “infant” or “child” or “adolescent” AND “weight gain” or “body mass index” or “rapid weight gain” or “linear growth” AND “glucose blood level” or “glucose tolerance test” or “glucose intolerance” or

“impaired fasting glycaemia” or “diabetes mellitus, type 2” or “hemoglobin A, glycosylated” or “insulin resistance”. These terms were selected in accordance with the Medical Subject Heading (MeSH) for the U.S National Library of Medicine, except “impaired fasting glycaemia” which was introduced as a text word. In addition, we also conducted a search of bibliographic references from articles finally selected from the search in online databases.

**Study selection and data extraction process**

Citations retrieved from online search were imported to EndNote X5 (Thomson Reuters, San Francisco, CA, USA) and duplicates were removed. Then, two authors (RB and MCR-M) screened the titles and examined the abstracts independently (Fig. 1). When agreement could not be reached by RB and MCR-M, a third author (ALGS) was consulted. Finally, full-text articles were scrutinized and data extraction was conducted.

Data on size effect and statistical significance (e.g., confidence interval or P-value) were required to analyze the studies.

**Data items**

Extracted characteristics included: first author's name, publication year, study design, setting, sample size, growth measures assessed, age at measurement, type of outcome, effect size and direction of the association (positive or negative), as well as covariates used in the adjustment.

**Risk of bias in individual studies**

Methodological study quality was assessed according to the Newcastle-Ottawa Scale (NOS) for cohort studies. This scale evaluates methodological rigor of observational studies assigning points in several questions organized in three categories: selection, comparability and ascertainment of the outcome. The maximum score is nine points, and we considered articles with a score of six or more as high quality.

### Synthesis of results

As the measures and periods of age of growth were heterogeneous among the studies, a meta-analysis was not considered appropriate. Thus, summary statistics were not estimated. Instead, a narrative approach was used to synthesize the study results.

We presented the results according to the outcomes, and we categorized the association between each period of growth and a specific outcome as "positive", "negative" or "no association".

## Results

### Study selection

We retrieved 4,876 records from our search strategy, of which 183 were duplicates. All article titles were screened and 70 remained for abstract selection. After reading abstracts, we selected 23 articles for full-text reading. From these, we selected 11 articles for narrative synthesis. Reasons for article exclusion included assessment of the exposure at a specific time point, specific population (e.g.: obese), outcome measured in adolescence and not being an original article (Fig. 1). No articles were selected from the bibliographic references of the selected articles.

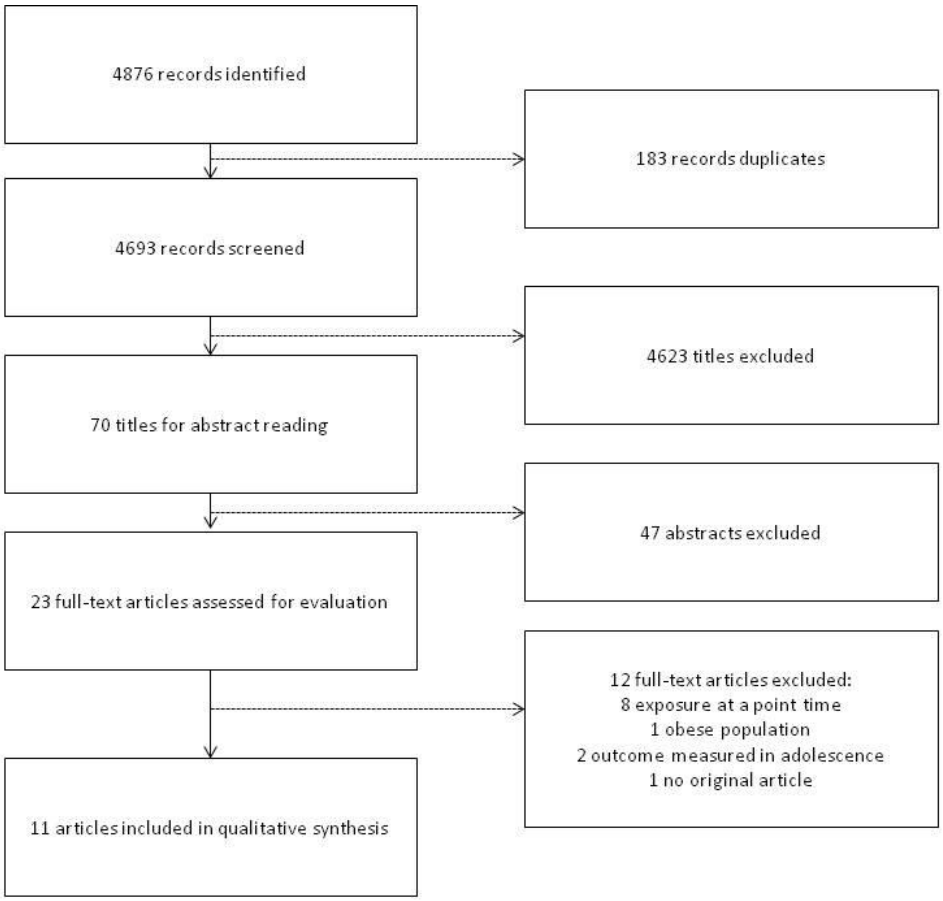


Figure 1. Search strategy and selection process

**Study characteristics**

Of the 11 studies included in this review, 10 were population-based cohorts and one was hospital-based cohort. Four studies were carried out in high-income countries and seven in low- and middle income settings, of which three analyzed pooled data from five low- and middle-income countries cohorts (Consortium of Health-Orientated Research in Transitioning Societies -COHORTS-). All the studies assessed both sexes and the sample size varied from 1,400 to 8,000, with the exception of Langeberg et al that evaluated a cohort of 282 individuals (Table 1).

Assessment of growth had great variation among studies in terms of definition, statistical methods and age intervals. The majority of the studies assessed growth during the first two years of life and childhood (between 2 and 11 years of age), and four of them also assessed growth between adolescence and the adult age when the outcome was measured. Specific age intervals from each study are listed in Table 1.

Five studies assessed growth using conditional measures based on standardized residuals of current and previous size measures. These conditional measures represent children's deviation from the expected size on the basis of their own previous measures and the mean growth of other children in the same cohort. This measure represents faster or slower relative weight gain or linear growth in relation to what is expected. These studies presented differences regarding age intervals measured and size parameters employed to examine growth (e.g. BMI, weight, length). (10-14). Among the remaining six studies, Power and Thomas examined BMI gain using a different conditional measure, where the increase of BMI z-score is interpreted as a rate of BMI change per 5 years for each interval of ages (15). Bhargava et al. examined changes in BMI through cross-tabulation trajectories of BMI z-score at specific points (e.g. 2 years, 12 years) (16). van de Langeberg et al. examined weight fluctuation through regression analyses using BMI z-score as the dependent variable and age as the independent variable, where the standard error around the regression coefficient represented the degree of weight fluctuation (17). Raghupathy et al. evaluated the change in BMI z-scores from birth, infancy, childhood or adolescence to adulthood (18). One study that analyzed data from a Finnish birth cohort applied complex statistical methods for modelling growth curves longitudinally from birth to age 2 years (19). Slining et al. estimated weight velocity as the change in weight between two measurements divided by the month interval between those measurements (20) (Table 1).

Eight studies assessed more than one outcome of interest (Table 1). These outcomes were measured among adults younger than 31 years old in most of the studies. Only one study analyzed middle-aged adults (45 years old) (15) and another one assessed individuals aged 55 to 66 years (12). All studies performed blood extraction to measure the outcomes, and dichotomous outcomes were mostly defined following international standard criteria or definitions. Although outcome scales varied among studies (e.g. score z, log transformation), the effect measurements used were mean differences or odds ratios. Details on the assessment and definition of outcomes are given in Supplementary Table A.1.

Table 1. Summary of the studies included in the narrative synthesis

| First author, year | Type of study<br>Setting | Sample size | Growth measurement and age intervals | Outcomes | Age at outcomes |
|---|---|---|---|---|---|
| Adair, 2013<br>(10) | COHORTS group<br>South Africa<br>Philippines<br>Brazil<br>India<br>Guatemala | 8362 | Conditional relative weight (CWh) and conditional height (CH)<br>at 2y,<br>at mid childhood (4, 5 or 8y),<br>at adulthood (18, 21, 23, 29 or 31y) | Fasting glucose<br>Dysglycaemia | 18/21/23/29/31<br>18 (South Africa),<br>21 (Philippines), 23 (Brazil), 29 (India), 31 (Guatemala) |
| Bhargava, 2004<br>(16) | New Delhi Birth cohort<br>India | 1526 | BMI trajectory between two points: 2y and 11y | IGT/DM | 31 |
| Eriksson, 2006<br>(12) | Helsinki University Central Hospital<br>Birth cohort | 2003 | Weight and BMI change<br>0 to 6 m<br>2 to 11y | Fasting glucose<br>2-h glucose after an OGTT<br>IGT | 56 to 66 |

| | | | | | |
|---|---|---|---|---|---|
| | Finland | | | DM<br>IGT/DM<br>HOMA-IR | |
| Fall, 2008<br>(13) | New Delhi Birth Cohort<br>India | 1583 | Weight and BMI change<br>0 to 2y<br>2 to 11y<br>11 to adult | Fasting glucose<br>2-h glucose after an OGTT<br>IGT/DM<br>HOMA-IR | 26 to 31 |
| Norris, 2012 (11) | COHORTS group<br>South Africa<br>Philippines<br>Brazil<br>India<br>Guatemala | 8746 | Conditional weight gain<br>0 to 2y<br>2 to 4y<br>4y to adult | Fasting glucose<br>IFG/DM<br>HOMA-IR | 15/21/23/29/31 15 (South Africa),21 (Philippines), 23 (Brazil), 29 (India), 31 (Guatemala) |
| Power, 2011 (15) | 1958 Birth Cohort<br>England, Scotland and Wales | 7855 | Conditional BMI change<br>0 to 7y<br>7 to 11y | Elevated HbA1c (>=7%) | 45 |

| | | | | | |
|---|---|---|---|---|---|
| | | | 11 to 16y | HbA1c (%) | |
| | | | 16 to 22y | | |
| | | | 23 to 33y | | |
| | | | 33 to 45y | | |
| | | | BMI change | | |
| Raghupathy, 2009 (18) | North Arcot Birth Cohort<br><br>India | 4092 | Birth to adulthood<br>Infancy (1-3m) to adulthood<br>Child (6-8y) to adulthood<br>Adolescent (10-14y) to adulthood | IGT/DM<br>Homa-IR | 28 |
| | COHORTS group | | | | |
| Stein, 2013 (14) | South Africa<br>Philippines<br>Brazil<br>India<br>Guatemala | 4518 | Conditional length/height<br>0 to 1y<br>0 to 2y<br>0 to mid childhood (4, 5 or 8y) | Fasting glucose<br>IFG/DM | 18/21/23/29/31<br>18 (South Africa), 21 (Philippines), 23 (Brazil), 29 (India), 31 (Guatemala) |

| | | | | | |
|---|---|---|---|---|---|
| Tzoulaki, 2010 (19) | Nothern Finland Birth Cohort 1966<br><br>Finland | 6007 | Peak weight (kg/year) and height (cm/year) velocity<br><br>0 to 2y | Fasting glucose | 31 |
| van de Langeberg, 2015 (17) | Prospective Terneuzen Birth Cohort<br><br>Netherlands | 282 | Weight fluctuation<br><br>2 to 6y | Fasting glucose | 18 to 28 |
| Slining, 2015 (20) | Cebu Longitudinal Health and Nutrition Survey<br><br>Philippines | 1409 | Weight velocity<br><br>0 to 4m<br><br>0 to 2y | Homa-IR | 21 |

COHORTS: Consortium of Health-Orientated Research in Transitioning Societies (prospective birth cohorts of low- and middle-income countries); DM: diabetes mellitus; IFG: impaired fasting glucose; Homa-IR: homeostasis model assessment for insulin resistance; HbA1c: glycated haemoglobin; IGT: impaired glucose tolerance; IGT/DM: combined prevalence of impaired glucose tolerance and diabetes mellitus; OGTT: oral glucose tolerance test

**Quality assessment risk of bias**

Based on the NOS, all included articles scored from 6 to 8 in terms of methodological quality (Supplementary Table A2). It is worth to notice that all studies scored zero in the item "demonstration that the outcome was not present at the start of the study". However, we decided to keep this item for informative purposes. The lowest scores were mainly due to lack of adjustment for birth weight or lack of inclusion of this variable in the growth measure, considered an important confounder in the relationship between growth and metabolic outcomes (5).

**Findings according to outcomes**

In order to summarize the main findings from the studies, we grouped the outcomes in four main categories: fasting blood glucose levels, 2-h glucose blood concentrations after an oral glucose tolerance test (OGTT), hyperglycaemia - which included impaired glucose tolerance, impaired fasting glucose, diabetes mellitus and elevated glycated haemoglobin-, and insulin resistance measured by homeostasis model assessment (HOMA-IR).

Fasting blood glucose levels

Seven studies examined the relationship between growth and fasting plasma glucose levels (Table 2) (10, 12-14, 17, 19). Adair et al. analyzed conditional height and relative weight at 2 years, during mid-childhood (four, five or eight years old depending on the follow-up of each cohort) and adulthood, and found that higher relative weight in adulthood was associated with greater values of fasting blood glucose at adult age in pooled analysis that included five cohort studies (10). In another analysis using the same population, Norris et al. found that conditional weight gain from 4 years old to adulthood was positively associated to fasting blood glucose levels. Eriksson et al found a negative association between weight change from birth to six months and adult fasting glucose concentrations when adjusted for current BMI (21). Fall et al. showed a positive association between BMI gain and weight gain from 11 years to adulthood and adult fasting glycaemia in India (13). van de Langeberg et al. compared young Dutch adults who belonged to the greatest weight fluctuation tertile and those with the lowest weight fluctuation tertile during childhood, and reported a lack of association with glycaemia (17). Similar findings were found when assessing weight peak velocity during the two first years of

life in a Finnish cohort (19). Linear growth during infancy, childhood and adolescence was not related to adult blood glucose levels in three studies either analyzed as conditional measures or as height velocity (10, 14, 19).

To summarize the evidence from these studies, we found no association between weight or BMI gain in the two first years of life and adult fasting glucose levels. However, excessive weight gain in later periods (mid-childhood and adulthood) was positively associated with fasting plasma glucose concentrations. In addition, we found no associations between linear growth and fasting glucose concentrations.

Table 2. Summary results for fasting blood glucose levels

| First author, year | Measure of growth and age interval | Association | Covariates |
|---|---|---|---|
| Adair, 2013* | Conditional relative weight (CWh) and conditional height (CH) at 2y, mid childhood (4, 5 or 8y) and adult age | Positive association: CWh-Adult | Sex, adult age, and site. |
| Eriksson, 2006 | Weight change (z-score) 0-6m | Negative association | Sex, age, and current BMI |
| | BMI change (z-score) 2-11y | No association | Sex and age |
| Fall, 2008 | BMI change and weight change 0-2y, 2-11y, 11y-adult | Positive association with 11y-adult | Sex, age, and lifestyle factors: alcohol consumption, physical activity, tobacco use, socioeconomic status in childhood -father's occupation-, in adult life -education level, household possessions and occupation-, family history of any of high blood pressure, angina, myocardial |
| Norris, 2012* | Conditional weight gain 0-2y, 2-4y, 4-adult | Positive association with 4-adult | Sex, adult age, and site |
| Stein, 2013* | Conditional length/ height at 1y, 2y, mid-childhood | No association | Site, sex, and adult age. |
| Tzoulaki, 2010 | Peak height (cm/year) and weight (kg/year) velocity in the first 2y | No association | Sex, gestational age, birth weight, adult BMI, maternal age, maternal height and weight before pregnancy, maternal smoking, and socioeconomic status at birth. |
| van de Langenberg, 2015 | Thirds of weight fluctuation 2-6y | No association | BMI-slope, birth weight, exclusive breastfeeding, maternal BMI, and educational level. |

* Except one of the sites: Brazil: random glucose adjusted for time since previous meal

BMI: body mass index; CH: conditional height; CWh: conditional relative weight

2 hours glucose concentrations after an oral glucose tolerance test (OGTT)

Adult blood glucose concentration after an OGTT was inversely related with weight change from birth to six months in the Helsinki birth cohort (12)and with BMI change during the first two years in the Indian cohort (13). In addition, adult blood glucose concentrations after an OGTT was positively associated with BMI gain and weight gain between 11 years and adulthood (13) (Table 3)

These findings suggest a negative association between growth during the first two years of life and adult blood glucose levels after an OGTT, as well as a positive association of growth during adolescence and adulthood and a 2-hour glucose concentration after an OGTT.

Table 3. Summary results for two-hour blood glucose after a 75-g Oral Glucose Tolerance Test

| First author, year | Exposure<br>Measure of growth | Association | Covariates |
|---|---|---|---|
| Eriksson, 2006 | Weight change (z-score) 0-6m | Negative association | Age, sex, and current BMI |
| | BMI change (z-score) 2-11y | No association | Age and sex |
| Fall, 2008 | BMI and Weight change 0-2y, 2-11y, 11y-adulthood | BMI change: negative association 0-2y<br>BMI and weight: positive association 11y-adulthood | Age, sex, and lifestyle factors: alcohol consumption, physical activity, tobacco use, socioeconomic status in childhood -father's occupation-, in adult life - education level, household possessions and occupation-, family history of any of high blood pressure, angina, myocardial |

BMI: body mass index

Hyperglycaemia

The association between growth and hyperglycaemia was assessed by seven studies (Table 3). Adair et al. found a positive association between conditional weight at adult age and dysglycaemia (fasting glucose concentrations ≥ 6.1 mmol/L or taking drugs for diabetes). Similar results were found for fasting glycaemia (10). Bhargava et al. evaluated the association between BMI trajectory and adult impaired glucose tolerance (IGT) or diabetes mellitus (DM) and found that individuals with low BMI at age 2, irrespectively of the BMI status in adulthood, presented higher risk of having IGT or DM. High BMI status in adulthood irrespectively of the BMI at age 2 also showed increased risk of having IGT or DM (16).

Eriksson et al. examined childhood BMI and weight change in relation to IGT, type 2 diabetes and the combined prevalence of both conditions, and found that type 2 diabetes and the combined prevalence of IGT and type 2 diabetes were positively associated with BMI change between age 2 to 11 years, and negatively associated with weight change during the first six months of life, after adjustment of current BMI (12).

The study carried out by Power and Thomas showed an increased HbA1c at 45 years with positive BMI changes in all assessed periods from birth to adulthood (0 to 7 y, 7 to 11y, 11 to 16y, 16 to 23y, and 23 to 33y), except for the 33 to 45 y interval. The authors also studied HbA1c as a continuous variable, and found a positive association with BMI change in all age intervals assessed (data not shown) (15).

Regarding linear growth, a study found a positive relation between conditional length at two years old and adult impaired fasting glycaemia (IFG)/diabetes (14). Another study did not find any relation between conditional height in infancy, childhood or adulthood and dysglycaemia (10).

To sum up, the majority of the results show a positive association of growth during childhood and adolescence and adult hyperglycaemia measures, supporting above mentioned findings for blood fasting glucose and glucose concentrations after a OGTT.

Table 4. Summary results for hyperglycaemia (impaired glucose tolerance, type 2 diabetes, impaired fasting glucose, elevated glycated haemoglobin)

| First author, year | Specific outcome associated with hyperglycaemia | Exposure | Association | Covariates |
|---|---|---|---|---|
| Adair, 2013 | Dysglycaemia | Conditional relative weight and conditional height at 2y, MC, adult | Positive association CWh-adult | Adult age, sex, and site. |
| Bhargava, 2004 | IGT/DM | BMI trajectory (cross-tabulation) | Positive association lowest tertile of BMI at 2y independently of adult BMI.<br>Positive association in the highest category of adult BMI independently of BMI at 2y | Sex and current age. |
| Eriksson, 2006 | IGT/DM | Weight change (z-score) 0-6m | Negative association | Age and sex, and current BMI |
| | | BMI change (z-score) 2-11y | Positive association | Age and sex |
| Fall, 2008 | IGT/DM | BMI and weight change 0-2y, 2-11y and 11y-adult | Positive association BMI and weight change 2-11y and 11-adult | Age, sex, and lifestyle factors: alcohol consumption, physical activity, tobacco use, socioeconomic status in childhood -father's occupation-, in adult life -education level, household possessions and occupation-, family history of any of high blood pressure, angina, myocardial |
| Norris, | IGF/DM | Conditional weight gain 0- | Positive association 4y- | Sex, site, adult age, and adult |

| | | | | |
|---|---|---|---|---|
| 2012 | | 2y, 2-4y, 4y-adult | adult | WC. |
| Power, 2011 | Elevated HbA1c | Conditional BMI gain 0-7y, 7-11y, 11-16y, 16-23y, 23-33y, 33-45y | Positive association in all periods except the 33-45y | Sex, social class at childhood and adult age, family history of diabetes, ethnicity and baseline BMI |
| Stein, 2013 | IFG/DM | Conditional length/ height at 1y, 2y and mid-childhood | Positive association with conditional length 2y | Site, sex, and adult age. |

BMI: body mass index; CWh: conditional relative weight; DM: diabetes mellitus; HbA1c: glycated haemoglobin; IFG: impaired fasting glycaemia; IGT: impaired glucose tolerance, WC: waist circumference

HOMA-IR

Table 5 described studies that assessed the association between growth and HOMA-IR. Eriksson et al. showed an inverse association between weight change in the first six months of life and adult HOMA-IR, and a positive association of BMI change between two and 11 years old with HOMA-IR (12). Fall et al. reported a positive association between both BMI and weight change in all periods assessed (0 to 2, 2 to 11, and 11 to adulthood) and adult HOMA-IR, except for BMI change during the first two years of life (13). Norris et al. found that greater conditional weight gain in any period from birth to adulthood (0 to 2, 2 to 4, and 4 to adulthood) was associated to increased HOMA-IR (11). Positive associations were showed by Raghypathy et al, as well (18). Slining et al. used mediation analysis to test the relationship between greater weight velocity. from birth to 2 years and 2 to 4 years and higher adult HOMA-IR, and found an indirect association mediated through current BMI and waist circumference (WC) (20).

To conclude, these findings suggest that weight or BMI change through infancy, childhood and adolescence are positively associated with adult HOMA-IR index.

Table 5. Summary results for HOMA-IR

| First author, year | Exposure | Association | Covariates |
|---|---|---|---|
| Eriksson, 2006 | Weight change (z-score) 0-6m | Negative association | Age and sex and current BMI |
| | BMI change (z-score) 2-11y | Positive association | Age and sex |
| Fall, 2008 | BMI and weight change 0-2y, 2-11y, 11y-adult | Positive association with BMI and weight change in all periods, except BMI change 0-2y | Age, sex, and lifestyle factors: alcohol consumption, physical activity, tobacco use, socioeconomic status in childhood - father's occupation-, in adult life - education level, household possessions and occupation-, family history of any of high blood pressure, angina, myocardial |
| Norris, 2012 | Conditional weight gain 0-2y, 2-4y, 4y-adult | Positive association in all periods | Sex, site, adult age. |
| Raghupathy, 2009 | Change in BMI z-score birth/adult, Infancy (1-3m) adult, Child (6-8y)-adult, Adolescent(10-14y)-adult | Positive association in all periods | Age, sex, adult life style factors (area of residence, a family history of diabetes, household possessions, higher education level, alcohol intake, tobacco, physical activity) |
| Slining, 2015 | Weight velocity from 0 to 4 months and 0 to 24 months. | No direct or total association 0-4 m<br>Positive total association 0-24 m among men.<br>Positive indirect effects mediated through BMI and WC in both sexes (both periods) | Age, small for gestational age status, mother's height, parity, urbanicity, socioeconomic status. |

BMI: body mass index; HOMA-IR: Homeostasis model assessment of insulin resistance; WC: waist circumference.

Summary statistics (regression coefficients and 95% CI) for every outcome are presented in Supplementary Tables A3, A4, A5, A6.

**Discussion**

This review synthesizes the literature that has been published in the area of growth (change in weight, height or BMI) during infancy, childhood and adolescence and glucose and insulin metabolism in adulthood. Accordingly with the results, rapid weight or BMI gain from mid-childhood onwards are related with increased risk of glucose and insulin disturbances in adulthood. By contrast, there is no evidence of association between accelerated weight or BMI gain during the first 2 years of life and glucose metabolism, although there is evidence of weight/BMI gain during this period and insulin resistance. No association was showed for linear growth in any period of life and metabolic risk profile.

It is well known that accelerated weight gain in those who had intrauterine growth restriction or were undernourished in the first 2 years of life is related with chronic diseases (6). In line with this evidence, our review adds that independently of nutritional status in infancy, those who gain weight more rapidly than theirs pairs especially from mid-childhood onwards, have higher risk of adult adverse metabolic glucose profile.

The development of high BMI after infancy leads to a body composition characterized by higher fat mass in comparison to lean body mass (22) that predisposes to alterations in glucose and insulin metabolism. Supporting this theory, Adair et al found that rapid weight gain in the first 2 years of life was associated with adult fat-free mass more than fat mass, as gaining accelerated weight after this age showed opposite results (10).

In this review we observed that linear growth at any age interval from infancy to adulthood was not related with any of the metabolic disturbances assessed. There is existing literature showing positive association between linear growth with human capital outcomes (e.g.: employment and earnings (23), but not with metabolic risk. Interestingly Adair et al was the only study that assessed both components of growth, separating the effect of weight and linear growth, and confirmed the current evidence. The study showed associations between faster height gain at infancy and mid-childhood with attained adult stature and education (10). Differently of height gain, rapid weight gain is usually accompanied with fat mass increase.

**Strengths and limitations**

This review was carried out following a disciplined approach, reducing the potential subjectivity in our findings. To our knowledge, this is the first review assessing a large range of age intervals of growth and a variety of glucose and insulin metabolism indicators. Another important strength is we used an extensive search strategy to maximize the sensitivity of the search and the inclusion of all outcomes. We only found cohort studies, although we believe that this is not a source of bias since this type of design was the most suitable to assess the aim of the present review.

We observed a large amount of heterogeneity in the methods to analyze growth, making challenging to draw conclusions. Specifically, the lack of consistency in the exposures investigated in terms of age intervals and growth definitions limits further comparison between studies and makes a meta-analysis difficult to be carried out. While some papers examined accelerated weight gain using conditional measures, others assessed weight change over time through weight fluctuation for example. Although all the studies were classified as having high methodology quality, as indicated by the NOS rating scale, they did not take account of the same confounding factors, which may have led to differences in results. Furthermore, two studies controlled for adult BMI and waist circumference (11, 12), which is controversial, as adult size is a potential mediator of the relationship between growth and adult disease. Also, we can not rule out the possibility of publication bias, since only published studies we assessed by this review.

**Conclusion**

We have some evidence to conclude that faster weight or BMI gain from childhood onwards may lead to an impaired glucose and insulin metabolism in adulthood across a range of socioeconomic settings. This should draw the attention of health care professionals as is relevant to the development of actions to promote healthy eating and physical activity habits to control the excessive weight gain after the first two years of life.

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**Supplementary tables**

Supplementary Table A1. Definition and scales of outcomes

| Outcome | Author | Scale | Definition |
|---|---|---|---|
| Fasting blood glucose | Adair, 2013 | Logarithm | Blood glucose concentrations after overnight fast in all cohorts, except in Brazil, where was obtained a random whole-blood sample adjusted for time since previous meal. |
| | Norris, 2012 | Score z | |
| | Stein, 2013 | Mmol/L | |
| | Eriksson, 2006 | Geometric mean | |
| | Fall, 2008 | Score z | Blood glucose concentrations after overnight fast |
| | Tzoulaki, 2010 | Percent | |
| | Langeberg, 2015 | Mmol/L | |
| Dysglycaemia | Adair, 2013 | Binary | Glucose concentrations ≥ 6.1 mmol/L or taking drugs for diabetes |
| IGT/DM | Bhargava, 2004<br>Fall, 2008<br>Raghupathy, 2009 | Binary | IGT: fasting plasma glucose concentration <7.0 mmol/L and a 2-h post glucose load ≥7.8 mmol/L<br>DM: fasting glucose concentration ≥7.0 mmol/L or a 2-h post glucose load concentration ≥11.1 mmol/L |
| IGT/DM | Eriksson, 2006 | Binary | Based upon an OGTT<br>IGT: 2-h post glucose load ≥7.8 mmol/L<br>DM: 2-h post glucose load ≥11.1 mmol/L |
| IFG/DM | Norris, 2012 | Binary | IFG: fasting glucose concentration ≥6.1 and <7.0 mmol/L<br>DM: fasting glucose concentration ≥7.0 mmol/L |
| | Stein, 2013 | | Stein, 2013 defined DM as fasting glucose concentration ≥7.0 mmol/L or a reported previous medical diagnosis.<br>Brazil: random whole-blood sample adjusted for time since previous meal.<br>Philippines: glucose levels assayed from whole venous blood samples. 0.97mmol/L was subtracted to estimate the best equivalent to venous plasma. |
| HOMA-IR | Eriksson, 2006 | Geometric mean | HOMA calculator: (fasting glucose x insulin) / 22.5 |
| | Fall, 2008 | Score z | |
| | Norris, 2012 | Score z | |
| | Raghupathy, 2009 | Score z | |
| | Slining, 2015 | Logarithm | |
| | | Binary | IR: HOMA-IR > 4.65 |
| Elevated $HbA_{1c}$ | Power, 2011 | Binary | HbA1c >7% (3 SDs above the mean) or self-reported type 2 DM |

DM: diabetes mellitus; HbA1c: glycated haemoglobin; HOMA-IR: homeostasis-model assessment of insulin resistance; IFG: impaired fasting glucose; IGT: impaired glucose tolerance; IR: insulin resistance

Supplementary Table A2. Methodological quality of the papers assessed using the Newcastle-Ottawa Scale.

| | Selection (4 points) | | | | | Comparability (2 points) | | | Ascertainment (3 points) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **First author, Year** | **Representa-tiveness of the exposed cohort** | **Selection of the non-exposed cohort** | **Ascertainment of exposure** | **Demonstration that outcome of interest was not present at start of study** | **Subtotal** | **Study controls for age and sex** | **Birth weight*** | **Subtotal** | **Assessment of outcome** | **Was follow-up long enough for outcomes to occur** | **Adequacy of follow up of cohorts** | **Subtotal** | **Result** |
| Adair, 2013 | 1 | 1 | 1 | 0 | 3 | 1 | 1 | 2 | 1 | 1 | 1 | 3 | 8 |
| Bhargava, 2004 | 1 | 1 | 1 | 0 | 3 | 1 | 0 | 1 | 1 | 1 | 1 | 3 | 7 |
| Eriksson, 2006 | 1 | 1 | 1 | 0 | 3 | 1 | 0 | 1 | 1 | 1 | 1 | 3 | 7 |
| Fall, 2006 | 1 | 1 | 1 | 0 | 3 | 1 | 1 | 2 | 1 | 1 | 1 | 3 | 8 |
| Langeberg, 2015 | 1 | 1 | 1 | 0 | 3 | 0 | 1 | 1 | 1 | 1 | 0 | 2 | 6 |
| Norris, 2012 | 1 | 1 | 1 | 0 | 3 | 1 | 1 | 2 | 1 | 1 | 1 | 3 | 8 |
| Power, 2011 | 1 | 1 | 1 | 0 | 3 | 1 | 0 | 1 | 1 | 1 | 1 | 3 | 7 |
| Raghupathy, 2009 | 1 | 1 | 1 | 0 | 3 | 1 | 0 | 1 | 1 | 1 | 0 | 2 | 6 |
| Slining, 2015 | 1 | 1 | 1 | 0 | 3 | 1 | 1 | 2 | 1 | 1 | 1 | 3 | 8 |
| Stein, 2013 | 1 | 1 | 1 | 0 | 3 | 1 | 1 | 2 | 1 | 1 | 1 | 3 | 8 |
| Tzoulaki, 2010 | 1 | 1 | 1 | 0 | 3 | 1 | 1 | 2 | 1 | 1 | 1 | 3 | 8 |

* Birth weight was considered if the analysis was adjusted for birth weight or if the conditional growth equation included this measurement.

Table A3. Growth age intervals and regression coefficients for fasting blood glucose levels

| First author, year | Exposure | Age exposure | Effect β (95% CI) |
|---|---|---|---|
| Adair, 2013 | Conditional relative weight (CWh) | CWh-2y | 0.00 (-0.02; 0.03) |
| | | CWh-MC | 0.02 (0.00; 0.05) |
| | | CWh-Adult | 0.11 (0.08; 0.13) |
| | Conditional height (CH) | CH-2y | -0.01 (-0.01; 0.04) |
| | | CH-MC | 0.00 (-0.02; 0.02) |
| | | CH-Adult | 0.02 (-0.00; 0.04) |
| Eriksson, 2006 | Weight change (z-score) | 0-6m | -0.6% (-1.2; -0.1) |
| | BMI change (z-score) | 2-11y | 0.2% (-0.4; 0.8) |
| Fall, 2008 | BMI change | 0-2y | -0.02 (-0.08;-0.03) |
| | | 2-11y | 0.03 (-0.03; 0.09) |
| | | 11y-adult | 0.08 (0.02; 0.14) |
| | Weight change | 0-2y | 0.04 (-0.02; 0.10) |
| | | 2-11y | 0.04 (-0.01; 0.10) |
| | | 11y-adult | 0.07 (0.01; 0.13) |
| Norris, 2012 | Conditional weight gain | 0-2y | 0.010 (-0.01; -0.03) |
| | | 2-4 | 0.014 (-0.01; 0.04) |
| | | 4-adult | 0.096 (0.07; 0.12) |
| Stein, 2013 | Conditional length/ height | At 1y | 0.01 (-0.02; 0.04) |
| | | At 2y | 0.02 (-0.01; 0.05) |
| | | At mid-childhood (4, 5 or 8y) | 0.01 (-0.02; 0.04) |
| Tzoulaki, 2010 | Peak height (cm/year) velocity | 0-2 y | -0.37 (-0.99; 0.47) |
| | Peak weight (kg/year) velocity | 0-2 y | -1.82 (-7.88; -12.5) |
| Langenberg, 2015 | Thirds of weight fluctuation | 2-6 y | Third 2 vs Third1: 0.11 (-0.09; 0.31)<br>Third 3 vs Third 1: -0.07 (0.28; 0.14) |

* Except one of the sites: Brazil: random glucose adjustment for time since previous meal
BMI: body mass index; CH: conditional height; CWh: conditional relative weight; MC:

Table A4. Growth age intervals and regression coefficients for two-hour blood glucose after a 75-g Oral Glucose Tolerance Test

| First author, year | Exposure | Age exposure | Effect β (95% CI) |
|---|---|---|---|
| Eriksson, 2006 | Weight change (z-score) | 0-6m | -2.9% (-4.2; -1.6) |
| | BMI change (z-score) | 2-11y | 1.1% (-0.3; 2.6) |
| Fall, 2008 | BMI change | 0-2y | -0.08 (-0.14;-0.02) |
| | | 2-11y | 0.04 (-0.02; 0.09) |
| | | 11y-adult | 0.19 (0.13; 0.25) |
| | Weight change | 0-2y | -0.04 (-0.10; 0.02) |
| | | 2-11y | 0.06 (-0.00; 0.12) |
| | | 11y-adult | 0.17 (0.11; 0.23) |

BMI: body mass index

Supplementary TableA5. Growth age intervals and regression coefficients for hyperglycaemia

| First author, year | Specific outcome | Exposure | Age exposure | Effect OR (95% CI) |
|---|---|---|---|---|
| Adair, 2013 | Dysglycaemia | Conditional relative weight | CWh-2y | 0.95 (0.86; 1.04) |
| | | | CWh-MC | 1.08 (0.98; 1.18) |
| | | | CWh-Adult | 1.32 (1.20; 1.45) |
| | | Conditional height | CH-2y | 0.98 (0.89; 1.18) |
| | | | CH-MC | 0.94 (0.86; 1.03) |
| | | | CWh-Adult | 1.03 (0.93; 1.13) |
| Bhargava, 2004 | IGT/DM | BMI trajectory (cross-tabulation) | BMI < 15.0 at 2y and BMI >16.2 at 12y | 4.5 (1.6; 12.8) |
| | | | BMI < 15.0 at 2y and BMI 22.7-26.5 at 26 to 32 y | 3.3 (1.9;7.2) |
| | | | BMI < 15.0 at 2y and BMI >26.5 at 26 to 32 y | 4.6 (1.9; 11.2) |
| | | | BMI 15.0-16.1 at 2y and BMI >26.5 at 26 to 32 y | 2.6 (1.1; 6.2) |
| | | | BMI >16.1 at 2y and BMI >26.5 at 26 to 32 y | 3.1 (1.3; 7.2) |
| Eriksson, 2006 | IGT | Weight change (z-score) | 0-6m | 0.91 (0.82; 1.01) |
| | | BMI change (z-score) | 2-11y | 1.06 (0.96; 1.18) |
| | DM | Weight change (z-score) | 0-6m | 0.85 (0.74; 0.97) |
| | | BMI change (z-score) | 2-11y | 1.20 (1.06; 1.35) |
| | IGT/DM | Weight change (z-score) | 0-6m | 0.88 (0.80; 0.97) |
| | | BMI change (z-score) | 2-11y | 1.12 (1.03; 1.22) |
| Fall, 2008 | IGT/DM | BMI change | 0-2y | 0.86 (0.72; 1.03) |
| | | | 2-11y | 1.25 (1.05; 1.47) |
| | | | 11y-adult | 1.40 (1.18; 1.67) |
| | | Weight change | 0-2y | 0.94 (0.79; 1.13) |
| | | | 2-11y | 1.26 (1.06; 1.49) |
| | | | 11y-adult | 1.31 (1.11; 1.56) |
| Norris, 2012 | IFG/DM | Conditional weight gain | 0-2y | 0.982 (0.844; -0.988) |
| | | | 2-4 | 0.998 (0.908; 1.062) |
| | | | 4-adult | 1.321(1.220; 1.430) |
| Raghupathy, 2009 | IGT/DM | Change in BMI z-score | Birth-adult | 1.31 (1.20; 1.43) |
| | | | Infancy (1-3m)adult | 1.26 (1.15; 1.40) |
| | | | Child (6-8y)-adult | 1.47 (1.32; 1.63) |
| | | | Adolescent(10-14y)-adult | 1.56 (1.38; 1.77) |
| | | Cross tabulation | Third 1 childhood BMI z- | 5.9 (2.9; 11.8) |

| | | | | |
|---|---|---|---|---|
| | | | score and adult BMI ≥23 | |
| | | | Third 2 childhood BMI z-score and adult BMI ≥23 | 4.2 (2.1; 8.2) |
| | | | Third 3 childhood BMI z-score and adult BMI ≥23 | 3.8 (1.9; 7.3) |
| Stein, 2013 | IFG/DM | Conditional length/ height | At 1y | 0.95 (0.78; 1.16) |
| | | | At 2y | 1.37 (1.12; 1.67) |
| | | | At mid-childhood (4, 5 or 8y) | 0.96 (0.79; 1.17) |

BMI: body mass index; DM: diabetes mellitus; IFG: impaired fasting glycaemia, IGT: impaired glucose tolerance.

Supplementary Table A6. Growth age intervals and regression coefficients for HOMA-IR

| First author, year | Exposure | Age exposure | Effect β (95% CI) |
|---|---|---|---|
| Eriksson, 2006 | Weight change (z-score) | 0-6m | -5.5% (-7.9; -3.0) |
| | BMI change (z-score) | 2-11y | 5.4% (2.3; 8.6 ) |
| Fall, 2008 | BMI change | 0-2y | 0.04 (-0.02; 0.09) |
| | | 2-11y | 0.15 (0.09; 0.20) |
| | | 11y-adult | 0.45 (0.13; 0.25) |
| | Weight change | 0-2y | 0.08 (0.02; 0.13) |
| | | 2-11y | 0.15 (0.10; 0.20) |
| | | 11y-adult | 0.44 (0.39; 0.49) |
| Norris, 2012 | Conditional weight gain | 0-2y | 0.100 (0.066; 0.133) |
| | | 2-4 | 0.110 (0.077; 0.144) |
| | | 4-adult | 0.355 (0.323; 0.387) |
| Power, 2011 | Conditional BMI gain. | 0-7y | 1.75 (1.42–2.16) |
| | | 7-11y | 1.66 (1.45–1.90) |
| | | 11-16y | 2.06 (1.69–2.51) |
| | | 16-22y | 2.99 (2.31–3.87) |
| | | 23-33y | 4.67 (3.12–7.00) |
| | | 33-45y | 1.24 (0.74–2.07) |
| Raghupathy, 2009 | Change in BMI z-score | Birth-adult | 0.063 (0.03; 0.09) |
| | | Infancy (1-3m) adult | 0.082 (0.05; 0.12) |
| | | Child (6-8y)-adult | 0.130 (0.09; 0.17) |
| | | Adolescent(10-14y)-adult | 0.081 (0.04; 0.12) |

BMI: body mass index; HOMA-IR: Homeostasis model assessment of insulin resistance.

**Artigo 2 - Distribution of glycated haemoglobin according to early-life and contemporary characteristics in adolescents and adults without diabetes: the 1982 and 1993 Pelotas birth cohorts**

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# DISTRIBUTION OF GLYCATED HAEMOGLOBIN ACCORDING TO EARLY-LIFE AND CONTEMPORARY CHARACTERISTICS IN ADOLESCENTS AND ADULTS WITHOUT DIABETES: THE 1982 AND 1993 PELOTAS BIRTH COHORTS

## Short title: GLYCATED HAEMOGLOBIN IN ADOLESCENTS AND ADULTS WITHOUT DIABETES

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**Abstract**

**Aim** Glycated haemoglobin ($HbA_{1c}$), a marker of glucose control in individuals with diabetes mellitus, is also related with the incidence of cardiometabolic risk in populations free of disease. The aim of this study was to describe the distribution of $HbA_{1c}$ levels according to early-life and contemporary factors in adolescents and adults without diabetes mellitus.

**Methods** $HbA_{1c}$ was measured in adults aged 30 years and adolescents aged 18 years who are participants in the 1982 and 1993 Pelotas Birth Cohorts, respectively. Bivariate and multivariate analyses were performed to describe the $HbA_{1c}$ mean values according to early-life and contemporary characteristics collected prospectively since birth.

**Results** The distribution of the $HbA_{1c}$ was approximately normal in both cohorts, with a mean (SD) 5.10% (0.43) in the 1982 cohort, and 4.89% (0.50) in the 1993 cohort. $HbA_{1c}$ mean levels were significantly higher in individuals self-reported as black/brown skin color compared to those self-reported as white in both cohorts. Parental history of diabetes was associated with higher $HbA_{1c}$ mean in adults, while stunting at one year old presented an inverse relation with the outcome in adolescents. No other early and contemporary factors were associated with $HbA_{1c}$ levels in adults or adolescents.

**Conclusions** We found a consistent relationship between $HbA_{1c}$ and skin color in both cohorts. Further research is needed to understand the role of genomic ancestry on levels of $HbA_{1c}$ concentrations which may inform policies and preventive actions for diabetes mellitus and cardiometabolic risk**.**

## Introduction

Glycated haemoglobin ($HbA_{1c}$) indicates the average blood glucose level during the previous eight to twelve weeks and is positively related to the concentration of glucose in blood [1]. $HbA_{1c}$ is recognized as the best index for long-term glucose control in diabetic patients [1]. Also, it is regarded as a useful screening tool for detecting diabetes in general population [2, 3]. In epidemiological studies, $HbA_{1c}$ has been found to be associated with atherosclerosis [4, 5], incidence of diabetes [6], cardiovascular disease (CVD) [7-9], and all-cause mortality in adult population without diabetes [10-13].

The $HbA_{1c}$ measurement offers some advantages compared with other glycemic indicators. It can be performed at any time of the day, irrespective of fasting or feeding, and it is relatively cheap [2, 14]. Therefore, $HbA_{1c}$ is used to examine the disease risk associated to hyperglycaemia in healthy populations [15].

Birth weight and nutritional status in the two first years of life have been associated with different diseases as coronary heart disease, impaired glucose tolerance and diabetes ([16-18]. Alcohol and tobacco use, physical inactivity, obesity, high waist circumference are well known risk factors for chronic diseases as well ([19, 20]. With the increase in metabolic conditions and the use of in $HbA_{1c}$ as glycaemic indicator, a better understanding of the relation between $HbA_{1c}$ concentrations and demographic, socioeconomic, behavioral and anthropometric characteristics is needed. This knowledge may help to identify groups of the population with increased risk of cardiometabolic diseases. Although the levels of $HbA_{1c}$ have been described for adults and

adolescents without diabetes [21, 22], the literature examining these associations in healthy populations is still scant.

In this study we aimed to describe the distribution of $HbA_{1c}$ levels according to known early-life and contemporary cardiometabolic risk factors in adolescents and adults without diabetes who are participants of two population-based birth cohorts.

## Materials and Methods

### Study design and population

Pelotas is a middle-sized city located in the extreme south of Brazil. The estimated total population for 2014 was approximately 340,000 inhabitants. In 1982 and 1993, two birth cohort studies began, in which all the residents in the urban area of Pelotas municipality were eligible. Based on the high percentage of hospital deliveries in the city (always above 98%), all the city's hospitals were daily visited by trained fieldwork team members. The mothers were interviewed soon after delivery using a structured questionnaire. Non-hospital deliveries were also included in the cohorts, since mothers normally sought a maternity ward after delivery, and were thus recruited to the study at this stage. The non-response rate at recruitment in both cohorts was below 1% [23]. Since then, the cohort members have been interviewed numerous times. On each occasion pre-tested standardized questionnaires were used and specially trained interviewers and field workers examined the subjects. Further detailed description of the methodology has been published previously [24-27].

Assessment of the outcome ($HbA_{1c}$) relies on the last visit of each cohort, the 30 years follow-up for the 1982 Cohort and the 18 years follow-up for the 1993 Cohort, which were

carried out in 2012-13 and 2011-12 respectively. In the 30 years wave, 4321 members were located, of whom 3701 were interviewed. Those who completed the interviews, added to those known to have died (n=325), represented 68% of the original cohort. In the 18 years visit, 87% (n = 4563) of the original cohort was located, and 4106 of these were examined. Including those members known to have died (n = 164), the follow-up rate was 81,3%. These follow-ups were carried out at the university research clinic and included interview, physical examination, assessment of physical activity and collection of biological samples [28, 29].

Study protocols were approved by the Medical Research Ethics Committee of the Federal University of Pelotas. Verbal informed consent for participation in the study was provided by mothers until 1986 in the 1982 Cohort and in perinatal phase 1993 Cohort. Written full informed consent was obtained from parents (if the participant was under 18 years old) or by the participants themselves at the subsequent visits. A written consent was not obtained in the earlier phases of the 1982 and 1993 Pelotas cohort studies as in Brazil the written inform consent was established only in 1996 by the National Health Council. Nonetheless, before 1997, the research projects were built considering a previous national resolution (Brazilian Resolution No.01 of June 13th, 1988) and followed the main regulation of national and international codes.

Outcome measurement

Blood samples were drawn by venipuncture and collected in ethylenediaminetetraacetic acid (EDTA) collection tubes in the day of the visit. Exclusion criteria for doing the blood drawing included pregnancy or probable pregnancy. Whole blood spot samples were prepared by pipetting 50 µL on to the filter paper card (Protein Saver $^{TM}$903® card, Whatman) and kept at

room temperature for drying. After drying, the filter cards were kept in sealed foil bags including desiccant pouch and stored at -80°C.

$HbA_{1c}$ was measured on whole blood samples by ion-exchange high-performance liquid chromatography (HPLC) method. Dried whole blood spots were prepared by cutting 3mm punches from each filter paper card sample. The elution of the sample was performed according to the protocol used in the VARIANT$^{TM}$ II testing system (Bio-Rad Laboratories Inc, Hercules, CA). This analyzer was standardized by the Diabetes Control and Complications Trial reference method.The intraassay and interassay coefficients of variation were 0.9% and 3.4-5.1%, respectively. Results are shown in percentage of $HbA_{1c}$ of total hemoglobin.

We have information on $HbA_{1c}$ for 3543 participants in the 1982 Cohort (59.3% of the original cohort, and 96.7% of the followed-up in the 30 years visit); and 3831 in the 1993 Cohort (75% of the original cohort, and 93.3% of the followed-up in the 18 years visit). As we attempted to describe the distribution de HbA1c in population without diabetes, subjects on antidiabetic medication (oral hypoglycemic agents or insulin) and those who had values of Hba1c >= 6.5% (n= 36 and 26 in 1982 and 1993 cohorts, respectively) were excluded, which comprises the final sample included in our analyses.

Independent variables

Early-life variables

Self reported skin color was collected based on the categories proposed by the Brazilian Institute of Geography and Statistics: white, black, brown, yellow and indigenous; and then grouped as white or black/brown. The participants who described themselves as “yellow” or

"indigenous" were only 3% of the interviewed subjects and were removed from the analyses.

Birth weight, collected at perinatal follow-up, was measured by hospital staff using pediatric scales whose accuracy were periodically checked by the research team. Low birth weight (LBW) was defined as birth weight <2500 g.

Nutritional status indicators at one year old were based on data of subsamples of both cohorts. The 1982 cohort subsample included all infants who were born from January to April 1982 (n=1916). In the 1993 cohort, all low birth weight (<2500 g) children plus 20% systematic sample of all other newborns were included (n=1460). Supine length measurement was taken using AHRTAG portable infantometers with 1 mm precision (AHRTAG, London, UK), custom built for these studies. Weight was evaluated using Salter CMS mechanical scales with 25 kg maximum and 100 g precision (Salter, Tonbridge, United Kingdom). In both cohorts, scales were calibrated on a weekly basis using standard weights. Length-for-age, weight-for-length and body mass index-for-age *z*-scores were calculated according to the growth curves published by the World Health Organization in 2006 .Children with z-scores of length-for-age and weight-for-length below −2 were classified as stunting and wasting, respectively. Overweight was defined as body mass index-for-age above +2 standards deviations.

Contemporary variables

The contemporary factors were assessed at the last waves of each cohort (30 and 18 years), with exception of parental history of diabetes on the 1993 cohort that was evaluated at the 11-year visit. Parental history of diabetes was assessed in both cohorts asking if one or both

parents had diabetes. Monthly family income in Brazilian currency (categorized in tertiles) was used as a measure of socioeconomic position (SEP).

Behavioral characteristics included smoking status, alcohol consumption and physical inactivity. The regular alcohol consumption was measured in categories of number of daily drinks, with slight differences between both cohorts. While in the 1982 cohort the categories were 0-1, 2-3, 4-5, 6-7, 8 or more alcohol servings per day; in the 1993 cohort the responses were 0, 1-2, 3-4, 5-6, 7-8, and 9 or more. Physical activity was assessed using a validated questionnaire which includes questions related to mode of commuting to school and work, and activities practiced during leisure time. The total physical activity score was generated by the sum of minutes per week spent on leisure-time and commuting activities. Adolescents with physical activity practice below 300 minutes per week were considered inactive. For adults, the cut-off point to define physical inactivity was 150 minutes per week [30].

Current weight was measured to the nearest 0.1 kg on electronic scale TANITA (model BC-418 MA; Tanita, Tokyo, Japan). Standing height was assessed to the nearest 0.1 cm using a wall-mounted stadiometer (SECA 240; Seca, Birmingham, United Kingdom).Body mass index (BMI) was calculated by dividing weight by height squared ($kg/m^2$) and cutoffs were defined separately for adults (20 y or more) [30],and for adolescents (10-19 y) [31, 32]. Due to small proportion of observations in the underweight category (less than 2% in each cohort), it was combined with the normal weight group. Waist circumference, categorized in tertiles, was evaluated with individuals in standing position using a flexible 160 cm (precision: 1 mm) fiberglass measuring tape. The measurement was taken at the narrowest point of the torso. Participants were barefoot and wearing light clothing.

Statistical analyses

The distribution of $HbA_{1c}$ in the sample studies was described using mean, standard deviation (SD) and histogram, and verified by values of skewness and kurtosis. As $HbA_{1c}$ was sufficiently normally distributed in both samples any transformation was unnecessary.

In the bivariate analyses, we described the mean and standard error (SE) of $HbA_{1c}$ according to categories of the independent variables. T-test or analyses of variance (ANOVA) were used to test for the significance of the mean differences in $HbA_{1c}$ among categories. When appropriate, test for linear trends were performed. To assess the independent association of the outcome and each of the independent variables, multiple linear regression was performed. All independent variables were included in a fully-adjusted model regardless of their level of statistical significance in the bivariate analysis of the association with the outcome measure. The same confounding structure was used for adjustment to facilitate the comparison between cohorts, thus variables that show association for either both cohorts or for one of them were included in the multivariate models. Interaction between $HbA_{1c}$ and sex was tested. As there was no strong evidence of heterogeneity of $HbA_{1c}$ concentrations between boys and girls, the results are shown for both sexes together.

For the main analyses presented here, we only included individuals who had complete data on any variables included in the fully- adjusted models (restricted sample). To assess possible effects of missing data from loss to follow-up, unadjusted analyses were carried out comparing both the restricted sample used in the main analyses with the maximal sample available for each independent variable. Analysis using data of the 12-month follow-up of the

1993 were weighted to correct for the oversampling of low birth weight. All the analyses were performed using the software Stata version 12.1 (StataCorp, College Station, TX, USA).

**Results**

Table 1 shows the description of the study samples. About half of the members were girls, and approximately two thirds were white. About 8% of the participants had low birthweight. At one year old, 8% of the 1982, and 13% of the 1993 cohort had chronic malnutrition; while both cohorts presented approximately 2% of wasting and 10% of overweight. The prevalence of parental history of diabetes was about one third in the 1982 cohort and 8% in the 1993 cohort. Concerning smoking, about 24% and 14% of the adults and adolescents were smokers, respectively. In both cohorts, about 7% of the participants drunk nine or more alcohol servings per day, and about 40% were physically inactive. The prevalence of overweight and obesity was observed in more than a half of the 1982 cohort members, and less than one third in the 1993 cohort.

The distribution of independent variables in the total number of cohort members who participated in the last visits is shown in SI Table. The prevalence of low birth weight (7%) and men (49.3%) in the 1982 cohort are slightly underrepresented in the study sample compared with the total number of participants at the last visit (9% and 51.4% for low birth weight and men, respectively). No other differences were found between these samples characteristics in the cohorts..

**Table 1.** Characteristics of participants with information on normal values of $HbA_{1c}$*. 1982 and 1993 Pelotas Birth Cohorts.

| Independent variables | 1982 cohort | | 1993 cohort | |
|---|---|---|---|---|
| | N | % | N | % |
| **Early-life characteristics** | | | | |
| Sex | | | | |
| Girls | 1778 | 50.7 | 1892 | 49.7 |
| Boys | 1729 | 49.3 | 1913 | 50.3 |
| Skin Color | | | | |
| White | 2664 | 78.5 | 2340 | 66.4 |
| Black and brown | 731 | 21.5 | 1182 | 33.6 |
| Low birth weight | | | | |
| No | 3256 | 92.9 | 3459 | 90.9 |
| Yes | 250 | 7.1 | 346 | 9.1 |
| Stunting (<-2 height/age)** | | | | |
| No | 863 | 92.2 | 992 | 87.2 |
| Yes | 73 | 7.8 | 135 | 12.8 |
| Wasting (<-2 weight/height)** | | | | |
| No | 923 | 98.6 | 1407 | 99.0 |
| Yes | 13 | 1.4 | 10 | 1.0 |
| Overweight (>2 bmi/age)** | | | | |
| No | 874 | 93.4 | 961 | 90.9 |
| Yes | 62 | 6.6 | 96 | 9.1 |

| | | | | |
|---|---|---|---|---|
| **Contemporary characteristics** | | | | |
| Parental history of diabetes*** | | | | |
| No | 1960 | 67.8 | 3317 | 91.7 |
| Yes | 933 | 32.3 | 301 | 8.3 |
| Family income (tertiles | | | | |
| 1 (poorer) | 1133 | 34.1 | 1282 | 33.6 |
| 2 | 1093 | 33.0 | 1243 | 32.9 |
| 3 (richest) | 1086 | 32.9 | 1280 | 33.5 |
| Smoking | | | | |
| Non-smokers | 2027 | 58.5 | 2954 | 77.7 |
| Ex-smokers | 614 | 17.7 | 312 | 8.2 |
| Smokers | 824 | 23.8 | 536 | 14.1 |
| Alcohol intake (servings per day) | | | | |
| 0 to 2 | 794 | 30.5 | 1832 | 65.0 |
| 3 to 8 | 1611 | 61.8 | 796 | 28.2 |
| 9 or more | 200 | 7.7 | 192 | 6.8 |
| Physical inactivity | | | | |
| No | 1992 | 58.0 | 2319 | 61.1 |
| Yes | 1444 | 42.0 | 1477 | 38.9 |
| Nutritional status | | | | |
| Underweight and normal | 1478 | 42.5 | 2752 | 72.8 |
| Overweight | 1204 | 34.6 | 651 | 17.2 |
| Obesity | 797 | 22.9 | 375 | 9.9 |

| | | | | |
|---|---|---|---|---|
| Waist circumference (tertiles) | | | | |
| 1 (lowest) | 1165 | 33.4 | 1269 | 33.4 |
| 2 | 1165 | 33.3 | 1266 | 33.4 |
| 3 (highest) | 1160 | 33.3 | 1259 | 33.2 |

* Less than 6.5% or taking antidiabetic medication

**One year old follow-up

*** 1993 cohort: eleven year old follow-up

The mean age was 30.2 years for the 1982 cohort participants and 18.5 years for the 1993 cohort members. The distributions of $HbA_{1c}$ levels were approximately normal in both cohorts with a higher mean among individuals from the 1982 cohort than among those from the 1993 cohort (5.10% vs. 4.89%; $p<0.001$). The dispersion of $HbA_{1c}$ values, as reflected by the standard deviations, was larger in the 1993 cohort compared with the 1982 cohort (0.43% vs. 0.50%).

1982 cohort

The association between $HbA_{1c}$ levels and early and contemporary factors for adults members of the 1982 cohort are shown in Table 2. No mean differences were found for any of the indicators of nutritional status at aged 1-year-old. On the other hand, higher $HbA_{1c}$ mean levels were found among black/brown compared with white (5.1 9 vs. 5.09 p=0.023), and among those whose mother and/or father had diabetes (5.19 vs. 5.08 p=0.006). Monthly familiar income and behavioral characteristics such as alcohol intake, smoking status and physical inactivity were not associated with $HbA_{1c}$ levels. $HbA_{1c}$ means increased progressively from the lowest to the highest categories of BMI (p=0.036), being those classified as obese who presented the highest

level of $HbA_{1c}$.

The unadjusted results using the maximum sample available (S2 Table) were compared with those presented here in the main analyses (only included individuals with complete data on outcome and all independent variables). In general, associations in the maximal sample were similar to those observed in the restricted sample, except for significant differences between $HbA_{1c}$ and the independent variables wasting at 1-year-old and current waist circumference.

After adjustment for all early and contemporary characteristics, only skin color and parental history of diabetes remained associated with HbA1c mean levels (p=0.044 and p=0.017, respectively).

**Table 2.** Unadjusted and adjusted mean and SE for $HbA_{1c}$ according to early-life, demographic, socioeconomic and behavioral factors, parent history of diabetes, nutritional status and waist circumference among adults in the 1982 Pelotas Birth Cohort.

| Independent variables | N | 1982 Cohort | | | |
|---|---|---|---|---|---|
| | | Unadjusted | | Adjusted | |
| | | Mean (SE) | p-value | Mean (SE) | p-value |
| **Early-life characteristics** | | | | | |
| Sex | | | 0.808 [a] | | 0.442 [c] |
| Girls | 291 | 5.10 (0.02) | | 5.10 (0.03) | |

| | | | | | |
|---|---|---|---|---|---|
| Boys | 270 | 5.11 (0.03) | | 5.13 (0.03) | |
| Skin Color | | | 0.023 [a] | | 0.044 [c] |
| White | 455 | 5.09 (0.02) | | 5.10 (0.02) | |
| Black and brown | 106 | 5.19 (0.04) | | 5.18 (0.03) | |
| Low birth weight | | | 0.308 [a] | | 0.249 [c] |
| No | 533 | 5.11 (0.02) | | 5.12 (0.02) | |
| Yes | 28 | 5.05 (0.10) | | 5.02 (0.08) | |
| Stunting (<-2 height/age)* | | | 0.294 [a] | | 0.347 [c] |
| No | 527 | 5.12 (0.02) | | 5.12 (0.02) | |
| Yes | 34 | 5.04 (0.07) | | 5.05 (0.08) | |
| Wasting (<-2 weight/height)* | | | 0.139 [a] | | 0.075 [c] |
| No | 554 | 5.11 (0.02) | | 5.11 (0.02) | |
| Yes | 7 | 5.34 (0.11) | | 5.40 (0.16) | |
| Overweight (>2 bmi/age)* | | | 0.927 [a] | | 0.668 [c] |
| No | 525 | 5.11 (0.02) | | 5.12 (0.02) | |
| Yes | 56 | 5.12 (0.07) | | 5.08 (0.07) | |
| **Contemporary characteristics** | | | | | |
| Parental history of diabetes | | | 0.006[a] | | 0.017 [c] |
| No | 399 | 5.08 (0.02) | | 5.09 (0.02) | |
| Yes | 162 | 5.19 (0.03) | | 5.18 (0.03) | |
| Family income (tertiles) | | | 0.972[a] | | 0.548 [c] |
| 1 (poorer) | 166 | 5.12 (0.03) | | 5.10 (0.03) | |
| 2 | 172 | 5.12 (0.03) | | 5.11 (0.03) | |

| | | | | | |
|---|---|---|---|---|---|
| 3 (richest) | 223 | 5.11 (0.02) | | 5.12 (0.03) | |
| Smoking | | | 0.483 [a] | | 0.410 [b] |
| Non-smokers | 341 | 5.11 (0.02) | | 5.10 (0.02) | |
| Ex-smokers | 100 | 5.09 (0.04) | | 5.11 (0.04) | |
| Smokers | 120 | 5.15 (0.04) | | 5.15 (0.04) | |
| Alcohol intake (servings per day) | | | 0.956 [a] | | 0.949 [c] |
| 0 to 1 | 181 | 5.10 (0.03) | | 5.12 (0.02) | |
| 2 to 7 | 339 | 5.11 (0.02) | | 5.11 (0.03) | |
| 8 or more | 41 | 5.12 (0.06) | | 5.11 (0.07) | |
| Physical inactivity | | | 0.239 [a] | | 0.220 [c] |
| No | 328 | 5.10 (0.02) | | 5.10 (0.02) | |
| Yes | 233 | 5.13 (0.03) | | 5.14 (0.03) | |
| Nutritional status | | | 0.036[b] | | 0.136 [b] |
| Underweight and normal | 228 | 5.08 (0.03) | | 5.05 (0.02) | |
| Overweight | 194 | 5.12 (0.03) | | 5.15 (0.02) | |
| Obesity | 139 | 5.17 (0.03) | | 5.17 (0.02) | |
| Waist circumference (tertiles) | | | 0.123 | | 0.476[b] |
| 1 (lowest) | 180 | 5.11 (0.03) | | 5.07 (0.05) | |
| 2 | 190 | 5.07 (0.03) | | 5.07 (0.03) | |
| 3 (highest) | 191 | 5.16 (0.03) | | 5.11 (0.04) | |

$HbA_{1c}$ shown as percentage of total haemoglobin

SE: standard error

Adjusted for all the independent variables

*Subsample at one year-old follow-up (1983)

[a] T test or ANOVA

[b] Linear trend

[c] Wald test

1993 cohort

Table 3 shows the association between $HbA_{1c}$ levels and early and contemporary factors among adolescents members of the 1993 cohort. $HbA_{1c}$ levels were not associated with sex, low birth weight, wasting and overweight. However, those classified as stunted at 1-year-old, showed lower mean levels of $HbA_{1c}$ in adolescence than those classified as not stunted (4.75 vs. 4.89 p=0.029) Mean $HbA_{1c}$ levels were higher among black/brown skin color compared with white skin color (4.95 vs. 4.86 p=0.016). There was no difference in $HbA_{1c}$ mean levels according to parental history of diabetes, tertiles of family income, alcohol drinking, smoking, physical inactivity and BMI categories. Mean $HbA_{1c}$ levels were linearly higher with increasing tertiles of waist circumference (p=0.008). In the multivariate model, only skin color and stunting remained associated with $HbA_{1c}$ concentrations (p=0.023 and p=0.013, respectively).

In the crude analyses using the maximal sample available for each independent variable (S3 Table), significant differences were found between $HbA_{1c}$ and sex, alcohol drinking and physical activity; while stunting was not related to the outcome.

**Table 3.** Unadjusted and adjusted mean and SE for $HbA_{1c}$ according to early-life, demographic, socioeconomic and behavioral factors, parent history of diabetes, nutritional status and waist circumference among adolescents in the 1993 Pelotas Birth Cohort.

| Independent variables | N | 1993 Cohort | | | |
|---|---|---|---|---|---|
| | | Unadjusted | | Adjusted | |
| | | Mean (SE) | p-value | Mean (SE) | p-value |
| **Early-life characteristics** | | | | | |
| Sex | | | 0.085[a] | | 0.054[c] |
| Girls | 352 | 4.86 (0.03) | | 4.83 (0.01) | |
| Boys | 355 | 4.92 (0.03) | | 4.93 (0.01) | |
| Skin Color | | | 0.016[a] | | 0.023[c] |
| White | 471 | 4.86 (0.02) | | 4.85 (0.01) | |
| Black and brown | 236 | 4.95 (0.03) | | 4.95 (0.02) | |
| Low birth weight | | | 0.289 [a] | | 0.082 [c] |
| No | 493 | 4.88 (0.02) | | 4.87 (0.02) | |
| Yes | 214 | 4.92 (0.04) | | 4.95 (0.04) | |
| Stunting (<-2 height/age)* | | | 0.029 [a] | | 0.013 [c] |
| No | 615 | 4.89 (0.02) | | 4.89 (0.02) | |
| Yes | 92 | 4.75 (0.06) | | 4.71 (0.07) | |
| Wasting (<-2 weight/height)* | | | 0.194[a] | | 0.296 [c] |
| No | 704 | 4.87 (0.02) | | 4.88 (0.02) | |
| Yes | 3 | 5.21 (0.26) | | 5.14 (0.25) | |

| | | | | | |
|---|---|---|---|---|---|
| Overweight (>2 bmi/age)* | | | 0.282 [a] | | 0.127 [c] |
| No | 640 | 4.89 (0.02) | | 4.89 (0.02) | |
| Yes | 67 | 4.82 (0.06) | | 4.80 (0.06) | |
| **Contemporary characteristics** | | | | | |
| Parental history of diabetes | | | 0.837[a] | | 0.776[c] |
| No | 649 | 4.89 (0.02) | | 4.89 (0.01) | |
| Yes | 58 | 4.91 (0.06) | | 4.93 (0.03) | |
| Family income (tertiles) | | | 0.483[b] | | 0.522[c] |
| 1 (poorer) | 218 | 4.92 (0.03) | | 4.90 (0.02) | |
| 2 | 240 | 4.86 (0.03) | | 4.87 (0.02) | |
| 3 (richest) | 249 | 4.91 (0.03) | | 4.89 (0.02) | |
| Smoking | | | 0.074[b] | | 0.170[c] |
| Non-smokers | 516 | 4.89 (0.02) | | 4.89 (0.01) | |
| Ex-smokers | 67 | 4.78 (0.06) | | 4.78 (0.03) | |
| Smokers | 124 | 4.84 (0.04) | | 4.88 (0.02) | |
| Alcohol intake (servings per day) | | | 0.420[a] | | 0.287 [b] |
| 0 to 2 | 445 | 4.91 (0.02) | | 4.91 (0.03) | |
| 3 to 8 | 214 | 4.85 (0.03) | | 4.84 (0.04) | |
| 9 or more | 48 | 4.88 (0.07) | | 4.82 (0.07) | |
| Physical inactivity | | | 0.506 [a] | | 0.388[c] |
| No | 443 | 4.90 (0.02) | | 4.87 (0.01) | |
| Yes | 264 | 4.87 (0.03) | | 4.88 (0.01) | |

| | | | | | |
|---|---|---|---|---|---|
| Nutritional status | | | 0.316[a] | | 0.586[b] |
| Underweight and normal | 520 | 4.87 (0.02) | | 4.90 (0.01) | |
| Overweight | 114 | 4.94 (0.05) | | 4.86 (0.03) | |
| Obesity | 73 | 4.93 (0.06) | | 4.80 (0.03) | |
| Waist circumference (tertiles) | | | 0.008[b] | | 0.093[b] |
| 1 (lowest) | 252 | 4.84 (0.03) | | 4.83 (0.02) | |
| 2 | 231 | 4.86 (0.03) | | 4.84 (0.02) | |
| 3 (highest) | 224 | 4.97 (0.03) | | 4.98 (0.02) | |

$HbA_{1c}$ shown as percentage of total haemoglobin

SE: standard error

Adjusted for all the independent variables

*Subsample at one year-old follow-up (1994)

[a] T testor ANOVA

[b] Linear trend

[c] Wald test

**Discussion**

We have described the distributions and mean values of $HbA_{1c}$ according to demographic, socioeconomic, behavioral and anthropometric characteristics in two nondiabetic population-based cohorts. Our results showed higher $HbA_{1c}$ means black/brown individuals in both cohorts. Besides, we observed positive association between $HbA_{1c}$ mean levels and having parental history of diabetes in adult members of the 1982 cohort; and being not stunting at 1 year

old in adolescents members of the 1993 cohort.

This study found higher $HbA_{1c}$ levels in the 30 years old cohort members compared with the 18 years old cohort members. The increase of $HbA_{1c}$ with ageing has been observed in other studies [33] [21]. Gulliford et al. observed a 0.12% increased in glycated haemoglobin every ten years of age in a population that included individuals aged 16 years or more [33]. Studies that assessed adult and elderly population showed a positive association $HbA_{1c}$ and age that remained significant after adjustment for BMI [21, 34]. However, the relationship between $HbA_{1c}$ and ageing in younger populations is controversial. [22, 35] . A study examined children and adolescents from four to seventeen years and demonstrated a linear association between $HbA_{1c}$ and age only in females and African-American males [35]; while other reported no linear trend in a sample with participants aged five to 24 years old [22]. A previous study carried out with a subsample of males members of the 1982 cohort when participants were 18 year old reported a mean $HbA_{1c}$ of 5.22% [36] , which it is higher than the one we found in this study in adolescents members of the 1993 cohort, and even higher than the current $HbA_{1c}$ concentrations in the male participants of the 1982 cohort. This difference may be explained by variation among different assay methods.

The positive association between black individuals and $HbA_{1c}$ among diabetics or those with glucose impaired tolerance is well established in the literature showing higher $HbA_{1c}$ concentrations in African Americans relative to non-Hispanic Whites [37-39]. Our results support the finding that skin color is related with $HbA_{1c}$ values even among young nondiabetic populations [22, 35], which it is in line with observational studies that examined adults without diabetes and found higher $HbA_{1c}$ means in black relative to white individuals [40, 41]. The

higher $HbA_{1c}$ mean levels in blacks versus whites persist even after adjustment for SEP has been reported in various studies as well. The skin color differences may reflect an adverse profile of parameters related to glucose homeostasis such as insulin sensitivity and secretion [42] or higher prevalence of type 2 diabetes mellitus among minorities groups [43]. Reasons for skin color differences in $HbA_{1c}$ concentrations remain unclear [44]. An important issue to be addressed in extensive studies is whether the observed skin color differences in levels of hemoglobin glycation reflect a greater risk for cardiometabolic diseases in black people compared with whites.

Low birthweight is known to be related with an adverse glucose and insulin profile in adult life [45], although, the relationship is less clear regarding $HbA_{1c}$. In line with our findings, most of studies did not find any association between birthweight and $HbA_{1c}$ in children [46], adolescents [36] and adults [47]; while a recent study carried out in English children found a 0.04% increase in $HbA_{1c}$ for every 100 g of lower birthweight [48]. The mentioned association appeared after adjustment for current height and became stronger with further adjustment for body fatness, indicating that the association depends on childhood size more than birthweight per se. On other hand, wasting and stunting at first year of life were associated with $HbA_{1c}$ mean levels. In the 1982 cohort we observed that those adults who were classified as wasted at first year of life, presented higher mean $HbA_{1c}$ levels. However, this association disappeared in the adjusted model which may be explained by the small prevalence of wasting (1.4%). In addition, we observed an inverse association between stunting at first year of life and $HbA_{1c}$ mean levels in adolescent members of the 1993 cohort, which it is an unexpected finding considering previous research on the developmental origins of cardiometabolic conditions. Studies suggested

that individuals who are small in the first years of life and subsequently put on weight rapidly present the greatest levels of risk for several metabolic conditions as coronary heart disease [49, 50], impaired glucose tolerance [51] and blood pressure [52]. We cannot ruled out the possibility of residual confounding as this result was not consistent to that observed for adults in the 1982 cohort.

Previous studies showed positive association with family history of diabetes, a well known risk factor for development of diabetes, in adults [21, 40] as well in adolescents [22]. This finding, which it is consistent with what we found in the 1982 cohort, indicate that there are possibly genetic factors involved in this relationship. However, non-significant results were found in the 1993 cohort. The discrepancy in findings for parental history of diabetes between both cohorts may reflect differences in the parent's age of the cohort members. In the 1993 cohort the variable was assessed when the adolescents were eleven years old, thus, the parents the cohort members were quite younger than parents from the 1982 cohort members (in which the question was asked at the age of 30). This may be related to the low diabetes prevalence observed among parents from the 1993 cohort and, as a result, to the absent of association with $HbA_{1c}$ levels in the adolescents.

No mean differences were observed between family income and $HbA_{1c}$ in the current analysis. Even with diverse socioeconomic indicators, other reports have also demonstrated weak relationship between SEP and $HbA_{1c}$ in adolescents [22, 35]. The lack of association between familiar income and $HbA_{1c}$ may be due to the fact that socioeconomic level could have a delayed effect on physical health in relation to chronic conditions. Therefore, their effects are not evident at early ages (e.g. younger than 35 years). It has been suggested that the effect of socioeconomic inequalities on biological outcomes emerge later in life, partly linked to different indirect

pathways [53]. These may also explain the lack of association between behavioral factors and $HbA_{1c}$. Further studies of these cohorts later in life will be needed to test this hypothesis.

Our findings are consistent with data from the NHANES III regarding the association between $HbA_{1c}$ levels and BMI which is not significant after controlling for covariates such as sex, race, maternal BMI and socioeconomic status [35]. On the other hand, BMI was shown to be an independent correlate of $HbA_{1c}$ concentrations in Japanese middle-aged and elderly population [54]. Furthermore, waist circumference have also been positively related with $HbA_{1c}$ in the Japanese study [54] and in the Bogalusa cohort in the United States [40]. We observed positive trends of $HbA_{1c}$ means according to increasing tertiles of waist circumference, however this association disappeared after adjustment for covariates. Again, this discrepancy of our results in relation to previous studies may be explained by age. We examined a younger population than Nguyen et al. which examined adults aged 32 to 40 years, and Yoshida et al. which assessed individuals aged 50 to 74 year. Given the importance of the waist circumference as a predictor of CVD and type 2 diabetes [55], the lack of statistical significance is not a reason to not give attention to the mean differences we have found.

Given that the present study had *a priori* samples sizes, the minimum detectable differences were calculated with a 5% of alpha error and 80% of power. Based on these parameters, the study would have sufficient power to detect differences of $HbA_{1c}$ levels between 0.08 and 0.45 for early-life factors, and 0.05 and 0.12 for contemporary variables. As a limitation, it is important to mention the fact that most of the associations may have been underpowered to detect the minimum mean differences of $HbA_{1c.}$ Furthermore, when we examined the unadjusted differences between the outcome and categories of the independent

variables in both the restricted and maximal samples, we found more significant associations using the maximal samples (i.e. not restricting results to only those with complete data in the outcome and all covariates) in the unadjusted analyses. This may add evidence to the hypothesis of low power due to smaller sample size. However, we cannot rule out the possibility of bias due to missing data.

The strengths of our report include the two large population-based cohorts in middle-income setting and the assessment several independent variables, including early and contemporary life factors. It is important to highlight that the $HbA_{1c}$ measurement was evaluated for nearly 60% of the 1982 cohort members and 75% of the 1993 cohort members, which are high percentages of follow-up.

To sum up, we showed representative data on $HbA_{1c}$ distributions among individuals of 18 and 30 years old without diabetes mellitus belonging to two cohorts of southern Brazil. We found normal distributions of $HbA_{1c}$ values and a consistent relationship between $HbA_{1c}$ and self-assessed skin color in both cohorts. These findings suggest that more research is needed to understand the role of genomic ancestry on levels of $HbA_{1c}$ concentrations.

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**Supporting information**

**S1 Table.** Characteristics of participants who were evaluated at the 30 and 18 years old visits, 1982 and 1993 Pelotas Birth Cohorts, respectively.

| Independent variables | 1982 cohort | | 1993 cohort | |
|---|---|---|---|---|
| | N | % | N | % |
| **Early-life characteristics** | | | | |
| Sex | | | | |
| Girls | 2876 | 48.6 | 2642 | 50.3 |
| Boys | 3037 | 51.4 | 2606 | 49.7 |
| Skin Color | | | | |
| White | 2817 | 78.6 | 2769 | 66.5 |
| Black and brown | 768 | 21.4 | 1395 | 33.5 |
| Low birth weight | | | | |
| No | 5375 | 91.0 | 4739 | 90.3 |
| Yes | 534 | 9.0 | 510 | 9.7 |
| Stunting (<-2 height/age)* | | | | |
| No | 1333 | 91.5 | 1179 | 86.6 |
| Yes | 124 | 8.5 | 182 | 13.4 |
| Wasting (<-2 weight/height)* | | | | |
| No | 1427 | 97.9 | 1344 | 98.7 |
| Yes | 30 | 2.1 | 17 | 1.3 |
| Overweight (>2 bmi/age)* | | | | |
| No | 1356 | 93.1 | 1231 | 90.4 |
| Yes | 101 | 6.9 | 130 | 9.6 |
| **Contemporary characteristics** | | | | |
| Parental history of diabetes** | | | | |
| No | 2053 | 67.7 | 3987 | 91.8 |
| Yes | 980 | 32.3 | 354 | 8.2 |
| Family income (tertiles | | | | |
| 1 (poorer) | 1191 | 34.2 | 1404 | 34.2 |

| | | | | |
|---|---|---|---|---|
| 2 | 1137 | 32.6 | 1336 | 32.5 |
| 3 (richest) | 1155 | 33.2 | 1366 | 33.3 |
| Smoking | | | | |
| Non-smokers | 2139 | 58.7 | 3179 | 77.5 |
| Ex-smokers | 647 | 17.7 | 354 | 8.6 |
| Smokers | 860 | 23.6 | 570 | 13.9 |
| Alcohol intake (servings per day) | | | | |
| 0 to 2 | 836 | 30.8 | 1954 | 64.8 |
| 3 to 8 | 1669 | 61.6 | 859 | 28.5 |
| 9 or more | 205 | 7.6 | 203 | 6.7 |
| Physical inactivity | | | | |
| No | 2091 | 58.0 | 2495 | 60.9 |
| Yes | 1515 | 42.0 | 1600 | 39.1 |
| Nutritional status | | | | |
| Underweight and normal | 1509 | 42.5 | 2881 | 72.7 |
| Overweight | 1228 | 34.6 | 680 | 17.2 |
| Obesity | 814 | 22.9 | 400 | 10.1 |
| Waist circumference (tertiles) | | | | |
| 1 (lowest) | 1189 | 33.3 | 1330 | 33.4 |
| 2 | 1192 | 33.4 | 1325 | 33.3 |
| 3 (highest) | 1186 | 33.3 | 1322 | 33.3 |

* One year old follow-up

** 1993 cohort: eleven year old follow-up

**S2 Table.** Unadjusted mean and SE for $HbA_{1c}$ according to early-life, demographic, socioeconomic and behavioral factors, parent history of diabetes, nutritional status and waist circumference among adults in the 1982 Pelotas Birth Cohort in the maximal sample.

| Independent variables | N | Mean (SE) | p-value |
|---|---|---|---|

| | | | |
|---|---|---|---|
| **Early- life characteristics** | | | |
| Sex | | | 0.058[a] |
| Girls | 1778 | 5.09 (0.01) | |
| Boys | 1729 | 5.11 (0.01) | |
| Skin Color | | | 0.039 [a] |
| White | 2664 | 5.09 (0.01) | |
| Black and brown | 731 | 5.13 (0.02) | |
| Low birth weight | | | 0.585 |
| No | 3256 | 5.10 (0.01) | |
| Yes | 250 | 5.11 (0.03) | |
| Stunting (<-2 height/age)* | | | 0.570 |
| No | 863 | 5.12 (0.14) | |
| Yes | 73 | 5.09 (0.05) | |
| Wasting (<-2 weight/height)* | | | 0.012 |
| No | 923 | 5.11 (0.01) | |
| Yes | 13 | 5.40 (0.07) | |
| Overweight (>2 bmi/age)* | | | 0.495 |
| No | 874 | 5.12 (0.01) | |
| Yes | 62 | 5.08 (0.06) | |
| **Contemporary characteristics** | | | |
| Parental history of diabetes | | | 0.003 [a] |
| No | 1960 | 5.09 (0.01) | |
| Yes | 933 | 5.14 (0.01) | |

| | | | |
|---|---|---|---|
| Family income (tertiles) | | | 0.445 [a] |
| 1 (poorer) | 1133 | 5.08 (0.01) | |
| 2 | 1093 | 5.10 (0.01) | |
| 3 (richest) | 1086 | 5.10 (0.01) | |
| Smoking | | | 0.687 [a] |
| Non-smokers | 2027 | 5.09 (0.01) | |
| Ex-smokers | 614 | 5.11 (0.02) | |
| Smokers | 824 | 5.10 (0.02) | |
| Alcohol intake (servings per day) | | | 0.082 [a] |
| 0 to 1 | 1847 | 5.08 (0.02) | |
| 2 to 7 | 558 | 5.11 (0.01) | |
| 8 or more | 200 | 5.10 (0.03) | |
| Physical inactivity | | | 0.498 [a] |
| No | 1992 | 5.09 (0.01) | |
| Yes | 1444 | 5.10 (0.01) | |
| Nutritional status | | | <0.001[b] |
| Underweight and normal | 1478 | 5.07 (0.01) | |
| Overweight | 1204 | 5.10 (0.01) | |
| Obesity | 797 | 5.15 (0.02) | |
| Waist circumference (tertiles) | | | <0.001[b] |
| 1 (lowest) | 1165 | 5.06 (0.01) | |
| 2 | 1162 | 5.10 (0.01) | |
| 3 (highest) | 1160 | 5.14 (0.01) | |

$HbA_{1c}$ shown as percentage of total haemoglobin

SE: standard error

Adjusted for all the independent variables

*Subsample at one year-old follow-up (1983)

[a] T test or ANOVA

[b] Linear trend

[c] Wald test

**S3 Table.** Unadjusted mean and SE for $HbA_{1c}$ according to early-life, demographic, socioeconomic and behavioral factors, parent history of diabetes, nutritional status and waist circumference among adolescents in the 1993 Pelotas Birth Cohort in the maximal sample.

| Independent variables | N | Mean (SE) | p-value |
|---|---|---|---|
| **Early-life characteristics** | | | |
| Sex | | | <0.001 [a] |
| Girls | 1892 | 4.83 (0.01) | |
| Boys | 1913 | 4.95 (0.01) | |
| Skin Color | | | 0.001 [a] |
| White | 2340 | 4.87 (0.01) | |
| Black and brown | 1182 | 4.92 (0.02) | |
| Low birth weight | | | 0.438 |
| No | 3462 | 4.89 (0.01) | |
| Yes | 347 | 4.91 (0.03) | |
| Stunting (<-2 height/age)* | | | 0.159 |

| | | | |
|---|---|---|---|
| No | 922 | 4.89 (0.02) | |
| Yes | 135 | 4.80 (0.06) | |
| Wasting (<-2 weight/height)* | | | 0.275 |
| No | 1047 | 4.88 (0.02) | |
| Yes | 10 | 5.07 (0.18) | |
| Overweight (>2 bmi/age)* | | | 0.387 |
| No | 961 | 4.88 (0.02) | |
| Yes | 96 | 4.84 (0.05) | |
| **Contemporary characteristics** | | | |
| Parental history of diabetes | | | 0.155 [a] |
| No | 3317 | 4.89 (0.01) | |
| Yes | 301 | 4.93 (0.03) | |
| Family income (tertiles) | | | 0.577[b] |
| 1 (poorer) | 1282 | 4.89 (0.01) | |
| 2 | 1243 | 4.90 (0.01) | |
| 3 (richest) | 1280 | 4.88 (0.01) | |
| Smoking | | | 0.698[b] |
| Non-smokers | 2954 | 4.89 (0.01) | |
| Ex-smokers | 312 | 4.89 (0.03) | |
| Smokers | 536 | 4.91 (0.02) | |
| Alcohol intake (servings per day) | | | 0.024 [a] |
| 0 to 2 | 1832 | 4.90 (0.01) | |
| 3 to 8 | 796 | 4.88 (0.02) | |

| | | | |
|---|---|---|---|
| 9 or more | 192 | 4.99 (0.03) | |
| Physical inactivity | | | 0.024 [a] |
| No | 2319 | 4.90 (0.01) | |
| Yes | 1477 | 4.87 (0.01) | |
| Nutritional status | | | 0.258 [b] |
| Underweight and normal | 2752 | 4.89 (0.01) | |
| Overweight | 651 | 4.90 (0.02) | |
| Obesity | 375 | 4.92 (0.03) | |
| Waist circumference (tertiles) | | | <0.001[b] |
| 1 (lowest) | 1269 | 4.85 (0.01) | |
| 2 | 1266 | 4.90 (0.01) | |
| 3 (highest) | 1260 | 4.92 (0.01) | |

$HbA_{1c}$ shown as percentage of total haemoglobin

SE: standard error

Adjusted for all the independent variables

*Subsample at one year-old follow-up (1994)

[a] T testor ANOVA

[b] Linear trend

[c] Wald test

**Artigo 3 - Growth across life course and cardiometabolic risk markers in 18 years old adolescents: the 1993 Pelotas Birth Cohort**

Será submetido para a revista *American Journal of Epidemiology*

As normas de publicação da revista podem ser acessadas através do seguinte *link*: https://academic.oup.com/aje/pages/Submission_Online

* Ver apêndice C para melhor entendimento das Tabelas 2 e 3 do artigo.

**Growth across life course and cardiometabolic risk markers in 18 years old adolescents: the 1993 Pelotas Birth Cohort**

Word count abstract: 242

Word count main text: 3367

Abstract

We aimed to evaluate the association between size at birth and growth trajectories from birth to adolescence and cardiometabolic risk markers levels at age 18 years. The outcomes were: random glucose, glycated hemoglobin (HbA1c), C-reactive protein (CRP), total cholesterol (TC), LDL-C, HDL-C, TGL, systolic and diastolic blood pressure (SBP and DBP), BMI and waist circumference (WC). Conditional relative weight (CWh) and conditional length/height (CH) were assessed using data from six follows-ups of the 1993 Pelotas Birth Cohort (at birth, 1, 4, 11, 15 and 18 years). In both sexes, greater CWh at 1 year was positively associated with BMI and WC, whereas greater CWh at most age periods in childhood and adolescence predicted increased values of CRP, TC, LDL-C, TGL, SBP, DBP, BMI and WC, and decreased HDL-C. Higher CH during infancy and childhood was positively related with SBP in boys and girls, and with BMI and WC only in boys. Our study showed that rapid weight gain from 1 year old onwards is positively associated with several markers of cardiometabolic risk at 18 years. The lack of anthropometric data at two years is an important limitation in our study, since there is evidence suggesting that the consequences of rapid weight gain on cardiometabolic health appear particularly after the two first years of life. Overall, our study support the "first 1000 days initiative" suggesting that prevention of excessive weight gain after age two years might be important in reducing later cardiometabolic risk.

Key-words: conditional growth, relative weight gain, linear growth, adolescents, cardiometabolic risk, cohort studies.

## Introduction

Metabolic and cardiovascular diseases (CVDs) are important public health problems, responsible for high morbidity and mortality burden in most regions of the world and causing enormous costs in terms of human and economic resources (1).

Growth trajectories throughout life course, including the fetal period, may have effects on cardiometabolic risk profile later in life (2). It is well established in the literature that low birthweight (a marker of fetal growth restriction) is a risk factor for type 2 diabetes (3), insulin resistance (4) and CVDs (5, 6). However, there is controversy about which age intervals of accelerated growth (weight and height changes) lead to the development of chronic conditions. Evidence suggests that accelerated weight gain during infancy is associated with an adverse metabolic risk profile at adulthood (7-9). In addition, it has been found that rapid weight gain increase the risk of metabolic disturbances when occurs during childhood, as a result of a more rapid development of body fat mass (10).

Studies on growth trajectories throughout life course and adult outcomes examine weight gain, without any distinction between the weight gain relative to height and linear growth. Dissimilar consequences of these measures have been found, which are relevant for healthcare policy makers when developing programs on cardiometabolic risk prevention. In this study, we aimed to assess the associations between size at birth (weight and length), conditional relative weight (CWh) and conditional length/height (CH) at ages 1, 4, 11, 15, and 18 years old and cardiometabolic risks markers (random glucose, glycated haemoglobin ($HbA_{1c}$), C-reactive protein (CRP), lipid profile, body mass index (BMI) and waist circumference (WC)) in adolescents aged 18 years old members of the 1993 Pelotas Birth Cohort study.

## Methods

The city of Pelotas is located in Southern Brazil. It is a middle-sized city with nearly 330,000 inhabitants. In 1993, all mothers of hospital-delivered newborns (99% of all births) who resided in the urban area of the city were invited to participate in a birth cohort study. Data were collected on 5,249 live births and only 16 individuals refused to participate. The cohort participants have been followed up at different time points thereafter. All visits were carried out by trained interviewers and fieldwork team members. Further details of the methodology have been published previously (12).

This study included information from six follow-ups: at perinatal, and at ages 1-, 4-, 11-, 15- and 18-years. The 1- and 4-year old follow-ups were conducted only in a subsample of all low birthweight children plus a random sample of 20% of the rest of the sample (1460 children). The response rates were 99.6%, 93.4%, 87.2% 87.5%, 85.2% and 81.4%, respectively Household visits were performed in every follow-up except for the 18-y old wave that took place at the university research clinic, where interviews, physical exams and collection of biological samples were carried out (13).

The study and its protocols were approved by the School of Medicine Ethics Committee of the Federal University of Pelotas. All participants or their legal representatives voluntarily signed a consent letter (verbal consent was provided in perinatal phase) prior to participation in each follow-up.

### Assessment of outcomes

We examined the following cardiometabolic risk markers measured at the 18-year old visit: random plasma glucose, $HbA_{1c}$, CRP, total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), triglycerides (TGL), systolic blood pressure (SBP), diastolic blood pressure (DBP), BMI and WC.

Random venous blood samples were collected, left at room temperature for 30 minutes and then centrifuged for 15 minutes at 2000g. Serum aliquots were stored at -80°C until analysis. The tests were not taken in pregnant or suspected pregnant. Glucose and lipids were measured using an automatic enzymatic colorimetric method in biochemistry analyzer BS-380, Mindray (Shenzhen Mindray Bio-Medical Electronics Co., Ltd, China). CPR was measured by turbidimetric immunoassay using also the BS-380 analyzer. $HbA_{1c}$ was measured on whole blood samples by ion-exchange high-performance liquid chromatography (HPLC) method using the VARIANT$^{TM}$ II system (Bio-Rad Laboratories Inc, Hercules, CA), which was standardized on Diabetes Control and Complications Trial standards.

Blood pressure was recorded in seated position using a calibrated digital wrist monitor (Omron HEM-629, Beijing, China). Measurements were taken at the start and at the end of the visit and mean of the two measurements was used in the analysis.

Current BMI was calculated by dividing weight in kilograms by height squared in meters. Weight was measured using a scale coupled to BodPod® equipment (Life Measurement, Inc., Concord, CA, USA) and height was obtained with standardized techniques using a wall-mounted stadiometer (SECA 240; Seca, Birmingham, United Kingdom). Waist circumference was measured with a fiberglass tape at the narrowest point of the torso.

**Assessment of exposures**

Birth weight and length were recorded in the maternity hospitals at delivery. In the subsequent follow-ups the measurements were conducted at participant's home, and at the research clinic in the 18-year old visit. On each occasion, weight and length or height were measured by trained field workers using standard equipment and protocols.

We studied birth and length at birth, and growth patterns in several life periods: infancy (from birth to 1 year old), early and mid-childhood (1 to 4 and 4 to 11 years old, respectively), and early and late adolescence (11 to 15 and 15 to 18 years old, respectively). For each age interval, the separate effects of weight gain and linear growth were examined, using conditional relative weight and conditional length/height proposed by Adair et al. Conditional relative weight takes into account current height and preceding weights and lengths or heights, and conditional length/height takes into account prior weight and length or height measures but not current weight (11).

To calculate these conditional measures, we computed sex-specific internal z-scores for weight and length or height at each follow-up. Then, we regressed the z-score size measurements (weight or height) at a given age, on z-scores at all previous measurements. The conditional measure is represented by the standardized residuals derived from the regression, and indicates the deviation from the individual's expected measures, in view of his or her previous growth and the average growth of the cohort members. This could be interpreted as a measure of relatively faster or slower weight or length/height change over a period of time. For example, an adolescence with a positive value in CWh from 11 to 15 years, put on greater weight compared with his or her previous weights and length/heights and weights and length/heights of all cohort

participants. As the conditional variables are uncorrelated, they can be included in a multiple regression model without breaking any assumption of collinearity (14).

**Statistical analyses**

We first described the outcomes by means (SD) (geometric means for CRP and TGL). T-tests were used to estimate mean differences between sexes. To assess the association between each outcome, weight and length at birth and conditional growth we used linear regression and p-values were obtained by Wald's test. The outcomes were standardized to allow direct comparisons of the regression coefficients. We adjusted for following confounder factors: family income (in minimum wages), maternal education (completed years of schooling) at birth and self-reported skin color (white, black or others). The random plasma glucose models were further adjusted for time from last meal (hours). Unadjusted and adjusted coefficients and statistical significance of associations I did not differ markedly with the inclusion of confounders in our models, thus we presented only adjusted results. Analyses were performed using Stata 12.1 (Stata Corp., College Station, Texas, and EUA) and stratified by sex.

**Results**

At a mean age of 18.5 years 4106 adolescents were evaluated, of which 3869 had blood information, 3987 had blood pressure measured, 3973 and 3977 had anthropometric exams (BMI and WC, respectively). Conditional relative weight and conditional length/height data across infancy, childhood and adolescence were available for 957 participants. Our main analysis

samples consisted of those cohort members who had complete data on exposures, confounders and at least one outcome (N= 917 for blood exams, and 946 for blood pressure, BMI and WC).

Mean levels of random glucose, $HbA_{1c}$, SBP were higher in boys than girls, as well as WC. On the other hand, girls had higher values on TC, HDL-C, LDL-C and TGL compared to boys. No differences were shown for DBP and BMI by sex (Table 1).

Mean outcome values of the main analysis samples were compared with mean outcome values of all participants who had information on blood tests, blood pressure and anthropometric measures. Only few differences were observed between both samples. Boys included in the main analyses had lower CPR and higher HDL-C values as compared to boys who were evaluated at the 18 follow-up. Among females, those included in the main analyses showed higher LDL-C than the total girls who were examined at the 18 follow-up. (Supplementary table 1). Proportions of males and females were slightly different in both samples, with males slightly sub-represented in the main analyses sample compared with the sample comprised by all participants who had information on blood tests, blood pressure and anthropometric measures (about 47.6% vs 49.8%).

Table 1. Mean (SD) for outcomes at 18 years of age in the main analyses samples, stratified by sex. 1993 Pelotas Birth Cohort[a]

| **Outcomes** | Boys<br>N=438 | Girls<br>N=479 | P-value |
|---|---|---|---|
| Plasma glucose (mg/dl) | 93.60 (20.88) | 88.41 (15.36) | <0.001 |
| HbA1c (%) | 4.97 (0.60) | 4.86 (0.50) | <0.001 |
| Protein-c (mg/L) [b] | 0.64 (3.10) | 1.35 (3.92) | <0.001 |
| Total cholesterol (mg/dl) | 151.31 (24.46) | 172.29 (30.35) | <0.001 |
| HDL cholesterol (mg/dl) | 52.78 (8.75) | 59.50 (10.54) | <0.001 |
| LDL cholesterol (mg/dl) | 83.55 (18.46) | 97.14 (25.42) | <0.001 |
| Triglycerides (mg/dl) [b] | 70.81 (1.46) | 74.79 (1.44) | 0.03 |
| Systolic blood pressure (mm/Hg) [c] | 130.35 (11.56) | 115.40 (10.04) | <0.001 |
| Diastolic blood pressure (mm/Hg) [c] | 70.54 (8.29) | 69.71 (7.83) | 0.12 |
| Body mass index [c] | 23.00 (4.25) | 23.58 (5.05) | 0.05 |
| Waist circumference [c] | 77.51 (9.71) | 73.74 (10.32) | <0.001 |

Data are arithmetic mean (SD) unless otherwise indicated

[a] Main analyses sample includes individuals with complete data on all growth measures, all confounders and at least one outcome.

[b] Geometric mean (SD)

[c] Boys N= 447 ; girls N=499

Tables 2 and 3 show the association of size at birth, conditional relative weight and conditional height with cardiometabolic risk markers at 18 years old. In boys and girls, birth and length at birth showed no associations with plasma glucose, $HbA_{1c}$, CRP, lipid profile, and SBP at 18 years old. In boys, weight and length at birth were positive associated with DBP, BMI and WC. In girls, weight and length at birth showed a positive association with CC, but only birthweight was related to BMI.

CWh and CH at all ages were unrelated to plasma glucose in both sexes, except for a positive association of this outcome with CWh in early adolescence (11 to 15 years) in girls (Table 3). Greater CWh and CH at age interval 1 to 4 years were positively associated with $HbA_{1c}$ concentrations in boys. In girls, positive associations between CWh gain and $HbA_{1c}$ and negative associations between CH gain and $HbA_{1c}$ were observed in the period between 4 and 11 years (mid-childhood).

CRP levels showed positive association with CWh during childhood and adolescence (from 1 to 4 years, 4 to 11 years and 11 to 15 years in both sexes, and 15 to 18 years only in girls). The association between CRP and weight gain appeared to be stronger and with an increasing trend across age periods in girls. Positive associations between CRP and CH between 1 and 4 years were found in both sexes (Tables 2 and 3).

Overall, lipid profile was associated with CWh gain during childhood and adolescence, especially in boys. CWh in the early childhood (1 to 4 years) was positive associated only with TGL, and CWh between 4 and 11 years was positively associated with LDL-C and TGL and negatively associated with HDL-C (Table 2). CWh during early and late adolescence (11 to 15 years and 15 to 18 years) were positively related with TC, LDL-C and TGL, and negatively related with HDL-C(Table 2). In girls, HDL-C was inversely associated only with CWh in mid-

childhood; whereas TC and LDL-C and TGL showed positive associations with CWh in late adolescence (Table 3).

Findings for SBP were similar for both sexes. However, DBP showed different patterns between sexes. Higher CWh during early and mid-childhood (1-4 y and 4-11y) and through early and late adolescence (11-15 y and 15-18y) was associated with higher SBP in both sexes, and with higher DBP only in girls. Regarding linear growth, conditional length gain during the first year of life and CH gain during early and mid-childhood (1-4 y and 4-11y) was related with increased levels of SBP in both sexes, whereas CH gain during early and mid-childhood were related with increased levels of DBP only in girls (Tables 2 and 3).

CWh in all age periods were associated with higher BMI and WC in boys and girls, with apparently larger coefficients after the first year of life (Table 2 and 3). CH between birth and age 1 year were positive related with BMI and WC only in boys. CH throughout early and mid-childhood (1-4 y and 4-11y) was positive related with BMI and WC in both sexes (Table 2 and 3). CWh was more strongly related to BMI and WC than linear growth.

Table 2. Association of weight and length at birth, conditional relative weight and conditional height with cardiometabolic risk markers at 18 years old in boys. 1993 Pelotas Birth Cohort.

| | Cardiometabolic risk markers | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Plasma glucose | HbA1c | CRP | TC | HDL-C | LDL-C | TGL | SBP | DBP | BMI | WC |
| Size at birth | | | | | | | | | | | |
| Birth | -0.03 | 0.01 | 0.00 | 0.01 | -0.00 | 0.01 | 0.01 | 0.03 | 0.06 | 0.13 | 0.16 |
| | (-0.09; 0.01) | (-0.04; 0.06) | (-0.03; 0.04) | (-0.03; 0.05) | (-0.04; 0.03) | (-0.03; 0.05) | (-0.01; 0.03) | (-0.01; 0.07) | (0.01; 0.11) | (0.09; 0.17) | (0.11; 0.20) |
| Length | -0.04 | 0.03 | -0.00 | -0.01 | -0.01 | -0.02 | 0.00 | 0.04 | 0.07 | 0.08 | 0.12 |
| | (-0.09; 0.01) | (-0.02; 0.08) | (-0.03; 0.03) | (-0.05; 0.03) | (-0.05; 0.03) | (-0.06; 0.03) | (-0.02; 0.02) | (-0.00; 0.08) | (0.02; 0.11) | (0.03; .012) | (0.07; 0.17) |
| Conditional relative weight | | | | | | | | | | | |
| CWh 0-1 y | -0.01 | 0.02 | -0.00 | 0.07 | 0.03 | 0.07 | 0.01 | 0.04 | 0.04 | 0.24 | 0.26 |
| | (-0.10; 0.08) | (-0.09; 0.12) | (-0.04; 0.04) | (-0.01; 0.14) | (-0.05; 0.10) | (-0.01; 0.14) | (-0.01; 0.03) | (-0.04; 0.11) | (-0.06; 0.13) | (0.17; 0.33) | (0.18; 0.35) |
| CWh 1-4 y | 0.02 | 0.12 | 0.08 | 0.04 | -0.03 | 0.05 | 0.04 | 0.09 | 0.06 | 0.43 | 0.41 |
| | (-0.07; 0.11) | (0.02; 0.23) | (0.03; 0.13) | (-0.04; 0.12) | (-0.11; 0.05) | (-0.03; 0.13) | (0.01; 0.06) | (0.01; 0.17) | (-0.04; 0.16) | (0.35; 0.51) | (0.33; 0.49) |
| CWh 4-11 y | 0.08 | -0.00 | 0.09 | 0.06 | -0.08 | 0.09 | 0.02 | 0.08 | 0.10 | 0.48 | 0.44 |
| | (-0.00. 0.17) | (-0.01; 0.09) | (0.05; 0.14) | (-0.01; 0.13) | (-0.15; -0.01) | (0.01; 0.16) | (0.01; 0.04) | (0.01; 0.16) | (0.01; 0.19) | (0.42; 0.53) | (0.38; 0.50) |
| CWh 11-15 y | 0.05 | 0.06 | 0.08 | 0.13 | 0.01 | 0.15 | 0.05 | 0.08 | 0.09 | 0.33 | 0.29 |
| | (-0.05; 0.17) | (-0.04; 0.17) | (0.02; 0.13) | (0.05; 0.20) | (-0.07; 0.09) | (0.07; 0.23) | (0.02; 0.07) | (0.01; 0.15) | (-0.01; 0.19) | (0.28; 0.38) | (0.24; 0.36) |
| CWh 15-18 y | 0.06 | -0.06 | 0.04 | 0.16 | -0.11 | 0.18 | 0.07 | 0.12 | 0.08 | 0.46 | 0.47 |
| | (-0.03; 0.15) | (-0.16; 0.05) | (-0.01; 0.09) | (0.08; 0.23) | (-0.19; -0.04) | (0.11; 0.26) | (0.05; 0.09) | (0.04; 0.19) | (-0.01; 0.18) | (0.44; 0.47) | (0.45; 0.50) |
| Conditional length/height | | | | | | | | | | | |
| CH 0-1 y | -0.10 | 0.03 | 0.00 | -0.01 | -0.01 | -0.00 | -0.01 | 0.14 | 0.08 | 0.10 | 0.20 |
| | (-0.20;0.00) | (-0.08; 0.13) | (-0.04; 0.05) | (-0.10; 0.07) | (-0.09; 0.07) | (-0.09; 0.08) | (-0.03; 0.02) | (0.06; 0.23) | (-0.02; 0.18) | (0.01; 0.19) | (0.10; 0.29) |
| CH 1-4 y | 0.04 | 0.15 | 0.05 | 0.06 | 0.03 | 0.05 | 0.02 | 0.09 | 0.04 | 0.18 | 0.24 |
| | (-0.06; 0.13) | (0.05; 0.26) | (0.01; 0.11) | (-0.02; 0.14) | (-0.05; 0.11) | (-0.04; 0.13) | (-0.01; 0.04) | (0.01; 0.18) | (-0.06; 0.14) | (0.09; 0.26) | (0.17; 0.33) |
| CH 4-11 y | 0.02 | -0.02 | -0.00 | 0.04 | -0.08 | 0.07 | 0.02 | 0.13 | 0.21 | 0.13 | 0.18 |
| | (-0.07; 0.11) | (-0.12; 0.08) | (-0.05; 0.04) | (-0.03; 0.12) | (-0.15; -0.01) | (-0.01; 0.15) | (-0.00; 0.04) | (0.05; 0.21) | (0.12; 0.30) | (0.07; 0.20) | (0.11; 0.25) |
| CH 11-15 y | -0.02 | 0.03 | -0.04 | -0.00 | 0.01 | 0.00 | -0.02 | 0.03 | 0.04 | -0.01 | 0.02 |
| | (-0.12; 0.07) | (-0.07; 0.14) | (-0.09; 0.01) | (-0.08; 0.07) | (-0.07; 0.09) | (-0.08; 0.08) | (-0.04; 0.00) | (-0.05; 0.11) | (-0.07; 0.14) | (-0.06; 0.04) | (-0.04; 0.08) |
| CH 15-18 y | -0.06 | 0.03 | -0.00 | -0.10 | -0.03 | -0.08 | -0.02 | 0.07 | 0.02 | -0.05 | 0.02 |
| | (-0.14; 0.04) | (-0.08; 0.13) | (-0.05; 0.05) | (-0.17; -0.02) | (-0.11; 0.04) | (-0.15; -0.00) | (-0.04; 0.00) | (-0.01; 0.15) | (-0.08; 0.12) | (-0.10; -0.01) | (-0.03; 0.08) |

HbA1c: glycated hemoglobin, CRP: reactive-C protein, TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TGL: triglycerides, DBP: diastolic blood pressure, SBP: systolic blood pressure, BMI: body mass index, CC: waist circumference, CWh: conditional relative weight, CH : conditional height

Data are β (95% CI). The outcome variables were normalize. Regression coefficient (β) values were calculated with linear regression models and indicate the SD change in the outcome per SD change in the predictor. All models were adjusted for mother's education (years of schooling) and household wealth (in minimum wages) at birth and skin color of the adolescent. The models for plasma glucose concentrations were further adjusted for time from previous meal.

Table 3. Association of Conditional relative weight and conditional height with cardiometabolic risk markers at 18 years old in girls. 1993 Pelotas Birth Cohort

| | Cardiometabolic risk markers | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Plasma glucose | HbA1c | CRP | TC | HDL-C | LDL-C | TGL | SBP | DBP | BMI | WC |
| Size at birth | | | | | | | | | | | |
| Weight | -0.02 (-0.06; 0.02) | -0.04 (-0.09; 0.02) | 0.02 (-0.05; 0.06) | -0.01 (-0.05; 0.04) | 0.02 (-0.03; 0.06) | -0.02 (-0.07; 0.03) | 0.04 (-0.03; 0.10) | -0.01 (-0.04; 0.02) | -0.03 (-0.08; 0.01) | 0.13 (0.08; 0.18) | 0.11 (0.06; 0.16) |
| Length | -0.02 (-0.05; 0.03) | -0.01 (-0.05; 0.03) | 0.00 (-0.05; 0.06) | -0.00 (-0.05; 0.05) | 0.01 (-0.04; 0.05) | -0.01 (-0.06; 0.04) | 0.04 (-0.03; 0.10) | 0.00 (-0.03; 0.03) | 0.00 (-0.04; 0.05) | 0.04 (-0.00; 0.09) | 0.06 (0.01; 0.10) |
| Conditional relative weight | | | | | | | | | | | |
| CWh 0-1 y | -0.04 (-0.12; 0.04) | 0.07 (-0.01; 0.16) | 0.03 (-0.05; 0.12) | -0.01 (-0.10; 0.09) | -0.04 (-0.13; 0.05) | -0.01 (-0.11; 0.10) | 0.01 (-0.03; 0.06) | 0.06 (-0.00; 0.13) | 0.03 (-0.06; 0.12) | 0.25 (0.15; 0.35) | 0.21 (0.12; 0.30) |
| CWh 1-4 y | 0.03 (-0.06; 0.11) | -0.05 (-0.14; 0.04) | 0.08 (-0.01; 0.18) | -0.02 (-0.12; 0.08) | -0.02 (-0.11; 0.08) | -0.03 (-0.14; 0.07) | 0.03 (-0.03; 0.08) | 0.12 (0.05; 0.18) | 0.12 (0.03; 0.23) | 0.56 (0.47; 0.65) | 0.45 (0.37; 0.54) |
| CWh 4-11 y | -0.01 (-0.09; 0.07) | 0.10 (0.02; 0.18) | 0.12 (0.03; 0.20) | -0.04 (-0.13; 0.06) | -0.15 (-0.24; -0.07) | 0.03 (-0.07; 0.13) | 0.00 (-0.02; 0.02) | 0.10 (0.04; 0.17) | 0.13 (0.04; 0.22) | 0.62 (0.55; 0.68) | 0.53 (0.47; 0.59) |
| CWh 11-15 y | 0.08 (0.02; 0.15) | 0.04 (-0.04; 0.12) | 0.14 (0.06; 0.24) | 0.00 (-0.09; 0.01) | -0.18 (-0.27; -0.10) | 0.05 (-0.05; 0.15) | 0.03 (0.01; 0.05) | 0.15 (0.09; 0.21) | 0.11 (0.03; 0.20) | 0.45 (0.40; 0.50) | 0.39 (0.34; 0.46) |
| CWh 15-18 y | 0.05 (-0.01; 0.12) | -0.02 (-0.11; 0.06) | 0.21 (0.12; 0.30) | 0.14 (0.04; 0.24) | -0.10 (-0.19; -0.02) | 0.20 (0.09; 0.29) | 0.03 (0.01; 0.05) | 0.16 (0.10; 0.22) | 0.13 (0.04; 0.22) | 0.51 (0.50; 0.53) | 0.47 (0.43; 0.50) |
| Conditional length/height | | | | | | | | | | | |
| CH 0-1 y | 0.01 (-0.07; 0.09) | -0.04 (-0.13; 0.03) | 0.02 (-0.07; 0.13) | 0.08 (-0.01; 0.17) | 0.17 (0.08; 0.26) | 0.03 (-0.07; 0.12) | -0.02 (-0.07; 0.02) | 0.09 (0.02; 0.15) | 0.01 (-0.07; 0.10) | 0.01 (-0.08; 0.11) | 0.08 (-0.00; 0.17) |
| CH 1-4 y | -0.03 (-0.12; 0.05) | 0.16 (0.08; 0.25) | 0.13 (0.03; 0.22) | 0.06 (-0.04; 0.16) | 0.11 (0.02; 0.21) | 0.01 (-0.09; 0.12) | 0.03 (-0.02; 0.08) | 0.10 (0.03; 0.17) | 0.11 (0.01; 0.20) | 0.12 (0.01; 0.22) | 0.22 (0.12; 0.31) |
| CH 4-11 y | -0.06 (-0.13; 0.02) | -0.10 (-0.17; -0.02) | -0.02 (-0.11; 0.07) | 0.05 (-0.04; 0.15) | 0.04 (-0.04; 0.13) | 0.04 (-0.06; 0.14) | -0.00 (-0.02; 0.02) | 0.08 (0.02; 0.14) | 0.20 (0.11; 0.29) | 0.12 (0.04; 0.20) | 0.12 (0.04; 0.20) |
| CH 11-15 y | 0.03 (-0.04; 0.09) | -0.07 (-0.15; 0.01) | -0.01 (-0.10; 0.08) | -0.02 (-0.11; 0.08) | -0.07 (-0.15; 0.02) | 0.02 (-0.09; 0.12) | -0.01 (-0.03; 0.02) | 0.08 (0.02; 0.15) | 0.02 (-0.07; 0.10) | -0.01 (-0.07; 0.06) | 0.08 (0.01; 0.13) |
| CH 15-18 y | 0.04 (-0.02; 0.11) | 0.05 (-0.03; 0.13) | -0.10 (-0.18; -0.01) | 0.00 (-0.09; 0.10) | -0.05 (-0.14; 0.03) | 0.01 (-0.09; 0.11) | 0.01 (-0.01; 0.03) | -0.00 (-0.07; 0.06) | 0.00 (-0.08; 0.09) | -0.05 (-0.07; -0.03) | -0.01 (-0.05; 0.05) |

HbA1c: glycated hemoglobin, CRP: reactive-C protein, TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TGL: triglycerides, DBP: diastolic blood pressure, SBP: systolic blood pressure, BMI: body mass index, CC: waist circumference, CWh: conditional relative weight =, CH : conditional height =

Data are β (95% CI). The outcome variables were normalized. Regression coefficient (β) values were calculated with linear regression models and indicate the SD change in the outcome per SD change in the predictor. All models were adjusted for mother's education (years of schooling), household wealth (in minimum wages) at birth and skin color of the adolescent. The models for plasma glucose concentrations were further adjusted for time from previous meal.

## Discussion

Our results showed some differences in the relative contributions of weight gain and linear growth to cardiometabolic risk. We observed that higher conditional relative weight at most age periods in childhood (1-4 y and 4-11 y) and adolescence (11-15 y and 15-18 y) was positively associated with most of the cardiometabolic markers (CRP, lipid profile, SBP, DBP, BMI and WC) at 18 years old adolescents of both sexes, while conditional relative weight during the first year of life was only related to BMI and WC. By contrast, associations between linear growth and the cardiometabolic markes showed a less consistent pattern when compared with weight gain. Greater conditional length at infancy was associated with higher values of SBP in boys and girls, and with BMI and WC only in boys.

Overall, our findings are consistent with published literature of low-, middle- and high-income countries showing that excessive weight gain after the second year of life predicts metabolic and cardiovascular diseases later in life (11, 15, 16). The association between weight gain throughout life and CRP concentrations at young adulthood was examined in the 1982 Pelotas Birth Cohort. In agreement with our analysis, the study showed that excessive weight gain at all ages periods after the second year of life in both sexes were positively associated with CRP levels in 23 years old participants of both sexes (17). We also found that excessive weight gain during childhood and adolescence was associated with increased total cholesterol, LDL-C and TGL and decreased HDL-C. Weight gain from birth to 4 years and lipid profile levels at 18 years was studied in the 1982 Cohort and negative associations were found between excessive weight gain from 2 to 4 years and HDL-C, although the association was reduced after controlling to current BMI (18). In a study using data from the ALSPAC cohort in England, the authors observed that greater BMI in mid-childhood predicted higher blood pressure (SBP and DBP) in 17 years old adolescents (19).

Fall et al., assessed the relations between components of metabolic syndrome and weight gain at three periods from birth to age 28 years, age in which the outcomes were measured. In line with our findings, the study showed that greater weight gain in childhood (2 to 11 years) was associated with increased values of WC, SBP and TLG, while greater weight gain from 11 to 28 years predicted higher WC, TGL, total cholesterol and SBP and lower HDL-C. They also observed positive associations between rapid weight gain during the two first postnatal years and WC, SBP and TGL (15).

In concordance with analysis from the Vellore Birth Cohort in India (20), we observed that infancy rapid conditional relative weight was positive associated with BMI and WC, but not with other cardiometabolic markers. A meta-analyses assessing infant growth and subsequent obesity, also showed that infants who grew more rapidly had higher risk of developing obesity at posterior ages (7). Studies that assessed the relation between growth and body composition showed that rapid weight gain in infancy were more related with fat mass than free fat mass in adulthood (11, 21). In contrast, rapid weight gain from childhood onwards generally is associated with accumulation of greater fat free mass compared with fat mass (10, 11, 21-23). These findings may explain the positive associations of relative weight gain at all age periods with BMI, as this indicator does not distinguish body fat from free fat mass.

In relation to linear growth, we observed that faster conditional height mainly in childhood was found to be positive associated with blood pressure (SBP and DBP), BMI and WC at 18 years old in both sexes, with less consistent associations in girls compared to boys. A previous report with data of the same cohort examined the association between conditional growth at three different age ranges up to 4 years old, BMI and blood pressure at 15 years, and found positive associations between conditional height from 1 to 4 years and both outcomes, although the association with SBP became insignificant adjusted for current BMI

(24). Haugaard et al, found positive association between linear growth during childhood and SBP and WC at age 8 years old (25). The previously mentioned Vellore Birth Cohort showed that rapid conditional height throughout course life was positive associated with blood pressure and WC in young adults (20). It is known that blood pressure is higher in taller people, this may be result of an adaptation of the vascular function to perfuse a longer arterial tree. However, inverse relations between adult height and cardiovascular diseases have been described as well (26), which suggests that this adaptation possibly has not pathological consequences.

Strengths of this study include the large and population based sample and the availability of several anthropometric and biological markers of cardiometabolic risk. Furthermore, our data have been collected prospectively since birth by trained staff with the use of standardized methods, reducing the susceptibility to misclassification. We also highlight the use of conditional growth to examine highly correlated measurements typical of longitudinal studies, and the assessment of the separate contributions of linear growth and weight gain relative to linear growth. Nevertheless, the assessment of relative weight gain does not distinguish between free-fat and fat mass gain.

We acknowledge some limitations of our study. First, at 18 years old, we followed up 81.3% of the original cohort and managed to have outcome measurements among most of them (94.2%, 97.1% and 97.8% for blood exams, anthropometric measures and blood pressure, respectively). These are high follow-ups rates for longitudinal studies and minimize the possibility of selection bias, however, our analysis were carried out only by those cohort members with anthropometric data from several follow-ups (including sub-samples). The potential impact of the losses on our results is difficult to assess. Second, we cannot rule out the possibility of random significant associations, as it is known that multiple hypothesis testing lead to increased Type I error. Third, measurements at age 2 years old were not

available in the 1993 Pelotas Birth Cohort, and therefore we had no ability to assess growth at this age point. The inclusion of age 2 years old in longitudinal studies assessing the associations between growth and cardiometabolic risk is very important due to growing evidence showing that excessive relative weight gain denotes increased risk for cardiometabolic health when occurred after the age of 2 years. For this reason, our findings support the initiative of improving nutrition during the "first 1000 days of life" (from conception up to age 2 years) to promote long-term benefits on health, as greater infant weight gain and linear growth have more benefits than risks for health, specially improvement of capital human outcomes as schooling and final achieved height (11, 16).

Given that cardiometabolic risk can track from adolescence to adulthood, a better understanding of the possible adverse effects of growth patterns at earlier stages in life can help to develop interventions aimed at preventing subsequent chronic diseases. Based on this study and other published evidence, we conclude that excessive weight gain from childhood onward may have an adverse effect on cardiometabolic health later in life. This reinforce efforts to inform strategies to avoid putting on weight children after their 2 first years of life for cardiovascular prevention. Evidence regarding linear growth throughout birth to late adolescence and subsequent cardiometabolic risk remains unclear and needs further investigation.

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## Supporting information

Table 1. Mean (SD) for outcomes at 18 years of age in the main analyses samples* and samples comprised by all the adolescents with outcomes measures at the 18 years follow-up, stratified by sex. 1993 Pelotas Birth Cohort.

| Outcomes | Boys | | | | | Girls | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | Main analyses sample | N | All participants | p-value* | N | Main analyses sample | N | All participants | p-value* |
| Plasma glucose (mg/dl) | 438 | 93.60 (20.88) | 1933 | 93.92 (22.65) | 0.79 | 479 | 88.41 (15.36) | 1936 | 89.74 (18.64) | 0.15 |
| HbA1c (%) | 438 | 4.97 (0.60) | 1924 | 4.96 (0.57) | 0.74 | 475 | 4.86 (0.50) | 1910 | 4.84 (0.52) | 0.45 |
| C-reactive Protein (mg/L) | 438 | 0.64 (3.10) | 1933 | 0.67 (3.26) | <0.01 | 479 | 1.35 (3.92) | 1936 | 1.35 (3.86) | 0.88 |
| Total cholesterol (mg/dl) | 438 | 151.31 (24.46) | 1933 | 152.72 (24.55) | 0.27 | 479 | 172.29 (30.35) | 1936 | 169.80 (29.19) | 0.10 |
| HDL cholesterol (mg/dl) | 438 | 52.78 (8.75) | 1933 | 51.78 (8.75) | 0.03 | 479 | 59.50 (10.54) | 1936 | 59.84 (10.92) | 0.54 |
| LDL cholesterol (mg/dl) | 438 | 83.55 (18.46) | 1933 | 84.28 (20.23) | 0.49 | 479 | 97.14 (25.42) | 1936 | 93.89 (23.84) | 0.01 |
| Triglycerides (mg/dl) | 438 | 70.81 (1.46) | 1933 | 73.56 (1.51) | 0.16 | 479 | 74.79 (1.44) | 1936 | 74.93 (1.49) | 0.62 |
| Systolic blood pressure (mm/Hg) | 447 | 130.3 (11.56) | 1979 | 130.71 (11.90) | 0.52 | 499 | 115.40 (10.04) | 2008 | 115.06 (9.95) | 0.50 |
| Diastolic blood pressure (mm/Hg) | 447 | 70.54 (8.29) | 1979 | 70.95 (7.94) | 0.33 | 499 | 69.64 (7.83) | 2008 | 69.46 (7.75) | 0.64 |
| Body mass index | 447 | 22.99 (4.24) | 1970 | 23.36 (4.23) | 0.10 | 499 | 23.58 (5.06) | 2003 | 23.52 (4.76) | 0.80 |
| Waist circumference (cm) | 447 | 77.51 (9.71) | 1972 | 78.45 (9.61) | 0.06 | 499 | 73.74 (10.31) | 2005 | 73.75 (9.75) | 0.98 |

Data are arithmetic mean (SD) unless otherwise indicated

* Geometric mean (SD)

*Main analyses samples includes individuals with complete data on all growth measures, all confounders and at least one outcome.

*p-value for T-test. C-reactive protein and triglycerides were log transformed to performed de test.

# COMUNICADO À IMPRENSA

**Ganhar peso de forma acelerada na infância e adolescência pode repercutir na saúde cardiovascular na vida adulta**

Um estudo da Universidade Federal de Pelotas revela que enquanto o ganho rápido de peso durante a infância e adolescência pode aumentar o risco cardiometabólico (risco geral de desenvolver diabetes e doenças cardiovasculares) na vida adulta, o ganho acelerado de estatura parece não ser prejudicial. "Indivíduos que engordaram rapidamente, especialmente depois dos dois anos de vida, tendem a apresentar níveis mais altos de colesterol no sangue, pressão arterial mais alta, assim como maior circunferência da cintura e do índice de massa corporal (IMC) que é obtido pela divisão do peso pela altura. Ao contrário, o ganho rápido de altura não traria consequências negativas à saúde cardiometabólica" afirma a nutricionista Romina Buffarini, autora da pesquisa publicada em tese de doutorado do Programa de Pós-Graduação em Epidemiologia do UFPel, sob orientação de Maria Cecília Formoso Assunção, María Clara Restrepo e Vera Maria Silveira.

O estudo incluiu dados de quase 1000 adolescentes que foram examinados pela equipe do Programa de Pós-Graduação em Epidemiologia desde o nascimento. Ao longo dos anos, os participantes do estudo foram medidos e pesados, e aos 18 anos, exames como aferição da pressão arterial, medição da circunferência da cintura, exames de sangue, foram realizados. Com os dados de peso e altura, os pesquisadores avaliaram o ganho de peso e altura dos participantes desde o nascimento até os 18 anos de idade e estudaram sua relação com alguns fatores de risco para doenças do coração e diabetes (doenças cardiometabólicas) aos 18 anos, como pressão arterial, circunferência de cintura, glicemia e colesterol em sangue, entre outros.

"A tendência normal de uma criança saudável é ganhar peso ao longo dos anos, porém, quando a gente fala de ganho rápido ou acelerado, está dizendo que a criança ganhou peso mais rapidamente do que deveria ter ganho em relação ao seu próprio ritmo de crescimento e ao ritmo de crescimento das outras crianças, com idade semelhante, que pertencem ao estudo. Este ganho de forma rápida, acarreta algumas alterações na saúde,, explica a autora. Para dar um exemplo, o nosso trabalho mostrou que aquele adolescente que entre os 4 e 11 anos ganhou peso mais rapidamente do que tinha ganho entre o nascimento e os 4 anos, tem níveis de colesterol no sangue um pouco aumentados em relação aos outros participantes do estudo que não tiveram este rápido ganho de peso. Neste sentido o nosso trabalho é muito importante pois faz um alerta de que engordar rapidamente nesta fase da vida pode ter consequências ruins para a saúde já no início da vida adulta.

A prevenção destas consequências deve incluir estratégias para evitar que crianças e adolescentes engordem, especialmente de forma  acelerada.

# ALTERAÇÕES SUGERIDAS PELA PRÉ-BANCA

Durante a avaliação desta tese por uma pré-banca, formada pelos examinadores internos (membros do Programa de Pós-graduação em Epidemiologia), novas análises foram sugeridas para o artigo 2, intitulado: "Distribution of Glycated Haemoglobin according to Early-Life and Contemporary Characteristics in Adolescents and Adults Without Diabetes: The 1982 and 1993 Pelotas Birth Cohorts", já publicado no periódico Plos One, em Setembro de 2016.

No manuscrito foram descritas as médias de HbA1c de acordo com variáveis sociodemográficas, comportamentais e de estado nutricional precoce e contemporâneo. As variáveis de estado nutricional precoce utilizadas (déficit de peso/idade, déficit de altura/idade e sobrepeso) são referentes aos acompanhamentos de 1 ano das coortes de 1982 e 1993, quando subamostras foram avaliadas. Sendo assim, o tamanho de amostra das análises (já publicadas) ficou consideravelmente reduzido. Novas análises foram realizadas com a exclusão destas variáveis. Também, foi proposto analisar os dados de acordo com modelos hierarquizados. Visto que o artigo já foi publicado, os resultados são apresentados no APÊNDICE B.

Outra sugestão da pré-banca foi mostrar as tabelas 2 e 3 do artigo 3 da tese (Growth across life course and cardiometabolic risk markers in 18 years old adolescents: the 1993 Pelotas Birth Cohort) de forma colorida para facilitar o entendimento das mesmas. As mesmas são apresentadas no APÊNDICE C.

# APÊNDICE A - COLETA DE SANGUE E DOSAGEM DA HBA1C

**Coleta de sangue e dosagem da HbA1c no acompanhamento dos 18 anos da coorte 1993 (2011/12) e acompanhamento dos 30 anos da coorte de 1982 (2012)**

1. Recrutamento

Para o cargo de coletador de sangue, o recrutamento foi feito separadamente pela pesquisadora bioquímica Isabel Oliveira (responsável técnica) e pela bióloga Helena Thurow. Foram entrevistadas 12 candidatas em 27 de julho de 2011. Os critérios para seleção foram: experiência em coleta de sangue, disponibilidade de horários, planos de futuros (cursos ou viagens), horários e dias de trabalho, salário e experiência no ramo.

2. Treinamento

Sete candidatos (enfermeiros e técnicos de enfermagem) foram selecionados para o treinamento. O treinamento para coleta, processamento, registro e armazenamento das amostras de sangue foi realizado nos dias 15 e 16 de agosto de 2011 em dois turnos (manhã e tarde) a fim de atender a disponibilidade de horários dos candidatos que trabalhavam em outros locais (hospitais e laboratórios de análises clínicas). No turno da manhã, dois candidatos receberam o treinamento, enquanto que no turno da tarde, cinco candidatos assistiram ao treinamento. A responsabilidade foi da pesquisadora Isabel Oliveira e da bolsista de pós doutorado Helena Thurow.

Para a coleta de sangue do acompanhamento da coorte 1982, ficou a mesma equipe, de sete pessoas, que havia trabalhado no acompanhamento da C93, não sendo necessário treinamento.

3. Coleta de sangue

Amostras de sangue aleatórias foram extraídas por punção venosa utilizando tubos de coleta de EDTA. A coleta era feita com o indivíduo deitado em uma maca. Eram coletados cinco tubos totalizando 20 mL de sangue. A ordem de coleta era: 1 – Tubo com gel e ativador de coágulo: 5 mL; 2 – Tubo com citrato de sódio: 2 mL; 3 – Tubo com EDTA: 4 mL; 4 – Tubo com gel e ativador de coágulo: 5 mL; e – Tubo com EDTA: 4 mL. O sangue coletado era levado para o laboratório de processamento no andar acima da clínica do CPE.

Mulheres grávidas ou possíveis grávidas foram critério de exclusão.

4. Descarte de material biológico

Os materiais biológicos e de consumo (ponteiras, tubos tipo falcon, luvas, agulhas, entre outros) provenientes da coleta e do processamento do sangue, bem como, da extração de DNA eram autoclavados antes do descarte (calor úmido: 15minutos, 120□C). A solução resultante da lise das hemácias realizada no protocolo de extração de DNA era armazenada em garrafas plásticas de 500 mL. Todo lixo contaminado era armazenado em sacos brancos leitosos (lixo hospitalar). O recolhimento desse lixo era realizado uma vez por semana, por uma empresa especializada, contratada pela Universidade, via Coordenadoria de Qualidade Ambiental, a qual era responsável pelo descarte por incineração.

5. Número de avaliados

Quadro 1. Número de adolescentes de acordo com a situação na coleta de sangue

| MATERIAL | REALIZOU | SEM AMOSTRA | TOTAL |
|---|---|---|---|
| Soro 0,5 mL | 3871 | 18 | 3889 |
| Soro 2 mL (alíquota A) | 3863 | 26 | 3889 |
| Soro 2 mL (alíquota B) | 3876 | 13 | 3889 |
| Soro com Glicerol | 3886 | 3 | 3889 |
| Plasma Citratado (alíquota A) | 3883 | 6 | 3889 |
| Plasma Citratado (alíquota B) | 3879 | 10 | 3889 |
| Plasma | 3884 | 5 | 3889 |
| Sangue – Papel Filtro | 3854 | 35 | 3889 |

6. Dosagem de HbA1c

A HBA1c foi dosada por cromatografia líquida de alta precisão (High-Performance Liquid Cromatography, HPLC) com o programa VARIANT II PROGRAMA HEMOGLOBINA A1C (Bio-Rad Laboratories Inc, Hercules, CA) a partir de amostras de 3 mm (punch of 3mm) obtidas do cartão de papel de filtro contendo sangue total coletado com EDTA. Foram usados 2 níveis de controles (LYPHOCHEK DIABETES) em cada corrida. Os coeficientes de variação intra-ensaio e inter-ensaio foram de 0,9% e 3,4-5,1%, respectivamente. Os resultados são apresentados na percentagem de HbA1c da hemoglobina total.

O sistema analítico do kit VARIANT$^{TM}$ II de instrumentos e reagentes proporciona um meio de medir a hemoglobina A1c, formada pela ligação não enzimática da glicose

sanguínea circulante à valina N-terminal da cadeia β da molécula de hemoglobina (HbAo). A ligação da glicose à hemoglobina é conseguida num processo de dois passos. O primeiro passo é a formação de uma aldimina instável (base de Schiff, lábil ou pré-A1c), uma reação reversível entre o grupo carbonila da glicose e a valina N-terminal da cadeia β da hemoglobina. A quantidade de base de Schiff formada é diretamente proporcional à concentração de glicose no sangue. O segundo passo é a conversão irreversível do intermediário de base de Schiff para uma cetoamina estável (Hemoglobina A1c). A percentagem de hemoglobina A1c no sangue total é dependente do nível de glicose sanguínea sustentada e indicativa da glicose sanguínea média ao longo da vida dos glóbulos vermelhos (≈120 dias).

# APÊNDICE B - ALTERAÇÕES SUGERIDAS PELA PRÉ-BANCA

## 1. Unadjusted and adjusted analyses with exclusion of nutritional status variables at 1 year old

In these new analyses, data on nutritional status (wasting, stunting and overweight) at 1 year old were excluded, consequently, sample size increased in the new analyses compared with the analyses from the published paper (from N=561 to N=2042 in the 1982 Pelotas Birth Cohort, from N=707 to N=2517 in the 1993 Pelotas Birth Cohort). The data was analyzed following the same statistical approach as in the published manuscript: fully-adjusted models (black-box) *(see Statistical analysis in Methods section from the published paper in page 123).*

***1982 cohort***

The association between $HbA_{1c}$ levels and early and contemporary factors for adults members of the 1982 cohort are shown in Table 1. In crude analyses, parental history of diabetes, alcohol intake, BMI and waist circumference were positive associated with $HbA_{1c}$. After adjustment for all characteristics, only parental history of diabetes remained associated with HbA1c mean levels.

**Table 1.** Unadjusted and adjusted mean and SE for $HbA_{1c}$ according to birth weight, parent history of diabetes and contemporary factor (demographic, socioeconomic and behavioral factors, nutritional status and waist circumference) among adults in the 1982 Pelotas Birth Cohort. N=2042

| Independent variables | N | 1982 Cohort | | | |
|---|---|---|---|---|---|
| | | Unadjusted | | Adjusted | |
| | | Mean (SE) | p-value | Mean (SE) | p-value |
| **Early-life characteristics** | | | | | |
| Sex | | | 0.159[a] | | 0.593[c] |
| Girls | 964 | 5.08 (0.01) | | 5.09 (0.02) | |
| Boys | 1078 | 5.11 (0.01) | | 5.11 (0.01) | |
| Skin Color | | | 0.105[a] | | 0.105[c] |

| | | | | | |
|---|---|---|---|---|---|
| White | 1633 | 5.09 (0.01) | | 5.09 (0.01) | |
| Black and brown | 409 | 5.13 (0.02) | | 5.13 (0.02) | |
| Low birth weight | | | 0.834[a] | | 0.783[c] |
| No | 1917 | 5.10 (0.01) | | 5.11 (0.01) | |
| Yes | 125 | 5.11 (0.04) | | 5.09 (0.04) | |
| **Contemporary characteristics** | | | | | |
| Parental history of diabetes | | | 0.011[a] | | 0.026[c] |
| No | 1392 | 5.08 (0.01) | | 5.08 (0.01) | |
| Yes | 650 | 5.13 (0.02) | | 5.13 (0.02) | |
| Family income (tertiles) | | | 0.355[a] | | 0.118[c] |
| 1 (poorer) | 640 | 5.08 (0.02) | | 5.07 (0.02) | |
| 2 | 652 | 5.11 (0.02) | | 5.11 (0.02) | |
| 3 (richest) | 750 | 5.10 (0.02) | | 5.11 (0.02) | |
| Smoking | | | 0.502[a] | | 0.632[c] |
| Non-smokers | 1182 | 5.09 (0.01) | | 5.09 (0.02) | |
| Ex-smokers | 356 | 5.11 (0.02) | | 5.11 (0.03) | |
| Smokers | 504 | 5.11 (0.02) | | 5.11 (0.02) | |
| Alcohol intake (servings per day) | | | 0.016[a] | | 0.051[c] |
| 0 to 1 | 641 | 5.06 (0.02) | | 5.06 (0.02) | |
| 2 to 7 | 1260 | 5.12 (0.01) | | 5.12 (0.01) | |
| 8 or more | 141 | 5.13 (0.04) | | 5.11 (0.04) | |
| Physical inactivity | | | 0.136[a] | | 0.125[c] |
| No | 1200 | 5.09 (0.01) | | 5.08 (0.01) | |
| Yes | 842 | 5.12 (0.02) | | 5.11 (0.01) | |
| Nutritional status | | | <0.001[b] | | 0.461[b] |
| Underweight and normal | 858 | 5.06 (0.01) | | 5.09 (0.02) | |
| Overweight | 715 | 5.11 (0.02) | | 5.10 (0.02) | |
| Obesity | 469 | 5.15 (0.02) | | 5.12 (0.03) | |
| Waist circumference (tertiles) | | | <0.001[b] | | 0.124[b] |
| 1 (lowest) | 653 | 5.05 (0.02) | | 5.06 (0.03) | |
| 2 | 672 | 5.10 (0.02) | | 5.10 (0.02) | |
| 3 (highest) | 717 | 5.15 (0.02) | | 5.13 (0.02) | |

$HbA_{1c}$ shown as percentage of total haemoglobin

SE: standard error

Adjusted for all the independent variables

*Subsample at one year-old follow-up (1983)

[a] T test or ANOVA

[b] Linear trend

[c] Wald test

***Comparison with published analyses***

The direction and significance of the associations between $HbA_{1c}$ and BMI, waist circumference and parental history of diabetes are similar in published and new analyses (unadjusted and adjusted models).

In the new analyses appeared a positive association between alcohol intake and $HbA_{1c}$.

Skin color, which was associated with $HbA_{1c}$ in the published manuscript, was not associated in the unadjusted or adjusted new analyses.

***1993 cohort***

Table 2 shows the association between $HbA_{1c}$ levels and early and contemporary factors among adolescents members of the 1993 cohort. $HbA_{1c}$ levels were associated with sex, skin color and waist circumference in crude and adjusted models.

**Table 2.** Unadjusted and adjusted mean and SE for $HbA_{1c}$ according to birth weight, parent history of diabetes and contemporary factor (demographic, socioeconomic and behavioral factors, nutritional status and waist circumference) among adolescents in the 1993 Pelotas Birth Cohort. N=2517

| Independent variables | N | 1993 Cohort | | | |
|---|---|---|---|---|---|
| | | Unadjusted | | Adjusted | |
| | | Mean (SE) | p-value | Mean (SE) | p-value |
| **Early-life characteristics** | | | | | |
| Sex | | | <0.001[a] | | <0.001[c] |
| Girls | 1241 | 4.84 (0.01) | | 4.86 (0.02) | |
| Boys | 1276 | 4.96 (0.01) | | 4.95 (0.01) | |
| Skin Color | | | 0.026[a] | | 0.028[c] |

| | | | | | |
|---|---|---|---|---|---|
| White | 1688 | 4.89 (0.01) | | 4.89 (0.01) | |
| Black and brown | 829 | 4.93 (0.02) | | 4.93 (0.02) | |
| Low birth weight | | | 0.766 [a] | | 0.484 [c] |
| No | 2288 | 4.80 (0.01) | | 4.90 (0.01) | |
| Yes | 229 | 4.91 (0.03) | | 4.92 (0.03) | |
| **Contemporary characteristics** | | | | | |
| Parental history of diabetes | | | 0.071[a] | | 0.129[c] |
| No | 649 | 4.90 (0.01) | | 4.89 (0.01) | |
| Yes | 58 | 4.96 (0.03) | | 4.95 (0.03) | |
| Family income (tertiles) | | | 0.981 [a] | | 0.983[c] |
| 1 (poorer) | 835 | 4.90 (0.02) | | 4.91 (0.02) | |
| 2 | 826 | 4.89 (0.02) | | 4.87 (0.02) | |
| 3 (richest) | 856 | 4.90 (0.02) | | 4.87 (0.03) | |
| Smoking | | | 0.311 [a] | | 0.251[c] |
| Non-smokers | 1875 | 4.90 (0.01) | | 4.91 (0.01) | |
| Ex-smokers | 232 | 4.87 (0.03) | | 4.87 (0.03) | |
| Smokers | 410 | 4.88 (0.02) | | 4.87 (0.03) | |
| Alcohol intake (servings per day) | | | 0.123[a] | | 0.195 [c] |
| 0 to 2 | 1640 | 4.90 (0.01) | | 4.91 (0.01) | |
| 3 to 8 | 714 | 4.89 (0.02) | | 4.88 (0.02) | |
| 9 or more | 163 | 4.98 (0.03) | | 4.95 (0.04) | |
| Physical inactivity | | | 0.051 [a] | | 0.193[c] |
| No | 1566 | 4.91 (0.02) | | 4.90 (0.01) | |
| Yes | 951 | 4.87 (0.02) | | 4.88 (0.02) | |
| Nutritional status | | | 0.199[b] | | 0.486[c] |
| Underweight and normal | 1825 | 4.89 (0.01) | | 4.91 (0.01) | |
| Overweight | 449 | 4.91 (0.02) | | 4.87 (0.03) | |
| Obesity | 243 | 4.94 (0.03) | | 4.89 (0.04) | |
| Waist circumference (tertiles) | | | <0.001[b] | | 0.022[b] |
| 1 (lowest) | 828 | 4.85 (0.02) | | 4.86 (0.02) | |
| 2 | 834 | 4.90 (0.02) | | 4.89 (0.02) | |
| 3 (highest) | 855 | 4.95 (0.02) | | 4.95 (0.02) | |

$HbA_{1c}$ shown as percentage of total haemoglobin

SE: standard error

Adjusted for all the independent variables

[a] T test of ANOVA

[b] Linear trend

[c] Wald test

***Comparison with published analyses***

The association between skin color and $HbA_{1c}$ concentration in the new analyses is similar to the analyses in the published paper. Regarding waist circumference, we found no association after adjustment for confounding factors which it is discordant with the published analyses.

The association between sex and $HbA_{1c}$ appeared only in the new analyses in both,unadjusted and adjusted models..

## 2. Hierarchical analyses

a) The following hierarchical model was assessed to analyzed data from the published manuscript:

| |
|---|
| Level 1<br>*Sex, skin color, parent history of diabetes* |
| Level 2<br>*Low birth weight* |
| Level 3<br>*Wasting, stunting, overweight at 1 year old* |
| Level 4<br>*Familiar income, smoking, alcohol intake, physical inactivity* |
| Level 5<br>*BMI , waist circumference* |
| Outcome<br>$HbA_{1c}$ |

The results were similar to those shown in the published manuscript *(see Results section in the published manuscript in pages 128 and 132).*

b) The following hierarchical model was assessed with data from new analyses:

| |
|---|
| Level 1<br>*Sex, skin color, parent history of diabetes* |
| Level 2<br>*Low birth weight* |
| Level 3<br>*Familiar income, smoking, alcohol intake, physical inactivity* |
| Level 4<br>*BMI , waist circumference* |
| Outcome<br>$HbA_{1c}$ |

The results were similar to those shown in the new analyses, following the fully-adjusted approach *(see Tables 1 and 2 in pages 184 to 187).*

In conclusion, for each sample size (published manuscript or new analyses), most of results are consistent in spite of the statistical approach used to analyze the data (fully-adjusted vs. hierarchical approach). However different findings related to sex and waist circumference were found in the 1993 cohort. Further analyses may be relevant to understand these differences.

# APÊNDICE C - TABELAS 2 E 3 DO ARTIGO 3 COLORIDAS

Tabelas 2 e 3 correspondentes ao artigo 3: "Growth across life course and cardiometabolic risk markers in 18 years old adolescents: the 1993 Pelotas Birth Cohort"

Conforme sugerido pela pré-banca, as tabelas 2 e 3 do artigo 3 da tese serão apresentadas de forma colorida para facilitar o entendimento das mesmas.

- Coeficientes positivos e seus intervalos de confiança 95% em cor azul.
- Coeficientes negativos e seus intervalos de confiança 95% em cor vermelha.
- Realce em amarelo para associações $p<0.05$.
- Realce em verde para associações $0.05 \leq p<0.10$.
- Realce em azul claro para associações $p \geq 0.10$.

Table 2. Association of weight and length at birth, conditional relative weight and conditional height with cardiometabolic risk markers at 18 years old in boys. 1993 Pelotas Birth Cohort.

| | Cardiometabolic risk markers | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Plasma glucose | HbA1c | CRP | TC | HDL-C | LDL-C | TGL | SBP | DBP | BMI | WC |
| Size at birth | | | | | | | | | | | |
| Birth | -0.03 (-0.09; 0.01) | 0.01 (-0.04; 0.06) | 0.00 (-0.03; 0.04) | 0.01 (-0.03; 0.05) | -0.00 (-0.04; 0.03) | 0.01 (-0.03; 0.05) | 0.01 (-0.01; 0.03) | 0.03 (-0.01; 0.07) | 0.06 (0.01; 0.11) | 0.13 (0.09; 0.17) | 0.16 (0.11; 0.20) |
| Length | -0.04 (-0.09; 0.01) | 0.03 (-0.02; 0.08) | -0.00 (-0.03; 0.03) | -0.01 (-0.05; 0.03) | -0.01 (-0.05; 0.03) | -0.02 (-0.06; 0.03) | 0.00 (-0.02; 0.02) | 0.04 (-0.00; 0.08) | 0.07 (0.02; 0.11) | 0.08 (0.03; .012) | 0.12 (0.07; 0.17) |
| Conditional relative weight | | | | | | | | | | | |
| CWh 0-1 y | -0.01 (-0.10; 0.08) | 0.02 (-0.09; 0.12) | -0.00 (-0.04; 0.04) | 0.07 (-0.01; 0.14) | 0.03 (-0.05; 0.10) | 0.07 (-0.01; 0.14) | 0.01 (-0.01; 0.03) | 0.04 (-0.04; 0.11) | 0.04 (-0.06; 0.13) | 0.24 (0.17; 0.33) | 0.26 (0.18; 0.35) |
| CWh 1-4 y | 0.02 (-0.07; 0.11) | 0.12 (0.02; 0.23) | 0.08 (0.03; 0.13) | 0.04 (-0.04; 0.12) | -0.03 (-0.11; 0.05) | 0.05 (-0.03; 0.13) | 0.04 (0.01; 0.06) | 0.09 (0.01; 0.17) | 0.06 (-0.04; 0.16) | 0.43 (0.35; 0.51) | 0.41 (0.33; 0.49) |
| CWh 4-11 y | 0.08 (-0.00; 0.17) | -0.00 (-0.01; 0.09) | 0.09 (0.05; 0.14) | 0.06 (-0.01; 0.13) | -0.08 (-0.15; -0.01) | 0.09 (0.01; 0.16) | 0.02 (0.01; 0.04) | 0.08 (0.01; 0.16) | 0.10 (0.01; 0.19) | 0.48 (0.42; 0.53) | 0.44 (0.38; 0.50) |
| CWh 11-15 y | 0.05 (-0.05; 0.17) | 0.06 (-0.04; 0.17) | 0.08 (0.02; 0.13) | 0.13 (0.05; 0.20) | 0.01 (-0.07; 0.09) | 0.15 (0.07; 0.23) | 0.05 (0.02; 0.07) | 0.08 (0.01; 0.15) | 0.09 (-0.01; 0.19) | 0.33 (0.28; 0.38) | 0.29 (0.24; 0.36) |
| CWh 15-18 y | 0.06 (-0.03; 0.15) | -0.06 (-0.16; 0.05) | 0.04 (-0.01; 0.09) | 0.16 (0.08; 0.23) | -0.11 (-0.19; -0.04) | 0.18 (0.11; 0.26) | 0.07 (0.05; 0.09) | 0.12 (0.04; 0.19) | 0.08 (-0.01; 0.18) | 0.46 (0.44; 0.47) | 0.47 (0.45; 0.50) |
| Conditional length/height | | | | | | | | | | | |
| CH 0-1 y | -0.10 (-0.20;0.00) | 0.03 (-0.08; 0.13) | 0.00 (-0.04; 0.05) | -0.01 (-0.10; 0.07) | -0.01 (-0.09; 0.07) | -0.00 (-0.09; 0.08) | -0.01 (-0.03; 0.02) | 0.14 (0.06; 0.23) | 0.08 (-0.02; 0.18) | 0.10 (0.01; 0.19) | 0.20 (0.10; 0.29) |
| CH 1-4 y | 0.04 (-0.06; 0.13) | 0.15 (0.05; 0.26) | 0.05 (0.01; 0.11) | 0.06 (-0.02; 0.14) | 0.03 (-0.05; 0.11) | 0.05 (-0.04; 0.13) | 0.02 (-0.01; 0.04) | 0.09 (0.01; 0.18) | 0.04 (-0.06; 0.14) | 0.18 (0.09; 0.26) | 0.24 (0.17; 0.33) |
| CH 4-11 y | 0.02 (-0.07; 0.11) | -0.02 (-0.12; 0.08) | -0.00 (-0.05; 0.04) | 0.04 (-0.03; 0.12) | -0.08 (-0.15; -0.01) | 0.07 (-0.01; 0.15) | 0.02 (-0.00; 0.04) | 0.13 (0.05; 0.21) | 0.21 (0.12; 0.30) | 0.13 (0.07; 0.20) | 0.18 (0.11; 0.25) |
| CH 11-15 y | -0.02 (-0.12; 0.07) | 0.03 (-0.07; 0.14) | -0.04 (-0.09; 0.01) | -0.00 (-0.08; 0.07) | 0.01 (-0.07; 0.09) | 0.00 (-0.08; 0.08) | -0.02 (-0.04; 0.00) | 0.03 (-0.05; 0.11) | 0.04 (-0.07; 0.14) | -0.01 (-0.06; 0.04) | 0.02 (-0.04; 0.08) |
| CH 15-18 y | -0.06 (-0.14; 0.04) | 0.03 (-0.08; 0.13) | -0.00 (-0.05; 0.05) | -0.10 (-0.17; -0.02) | -0.03 (-0.11; 0.04) | -0.08 (-0.15; -0.00) | -0.02 (-0.04; 0.00) | 0.07 (-0.01; 0.15) | 0.02 (-0.08; 0.12) | -0.05 (-0.10; -0.01) | 0.02 (-0.03; 0.08) |

HbA1c: glycated hemoglobin, CRP: reactive-C protein, TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TGL: triglycerides, DBP: diastolic blood pressure, SBP: systolic blood pressure, BMI: body mass index, CC: waist circumference, CWh: conditional relative weight gain, CH : conditional height gain

Data are β (95% CI). The outcome variables were normalize. Regression coefficient (β) values were calculated with linear regression models and indicate the SD change in the outcome per SD change in the predictor. All models were adjusted for mother's education (years of schooling) and household wealth (in minimum wages) at birth and skin color of the adolescent. The models for plasma glucose concentrations were further adjusted for time from previous meal.

Table 3. Association of Conditional relative weight and conditional height with cardiometabolic risk markers at 18 years old in girls. 1993 Pelotas Birth Cohort

| | Cardiometabolic risk markers | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Plasma glucose | HbA1c | CRP | TC | HDL-C | LDL-C | TGL | SBP | DBP | BMI | WC |
| Size at birth | | | | | | | | | | | |
| Weight | -0.02 (-0.06; 0.02) | -0.04 (-0.09; 0.02) | 0.02 (-0.05; 0.06) | -0.01 (-0.05; 0.04) | 0.02 (-0.03; 0.06) | -0.02 (-0.07; 0.03) | 0.04 (-0.03; 0.10) | -0.01 (-0.04; 0.02) | -0.03 (-0.08; 0.01) | 0.13 (0.08; 0.18) | 0.11 (0.06; 0.16) |
| Length | -0.02 (-0.05; 0.03) | -0.01 (-0.05; 0.03) | 0.00 (-0.05; 0.06) | -0.00 (-0.05; 0.05) | 0.01 (-0.04; 0.05) | -0.01 (-0.06; 0.04) | 0.04 (-0.03; 0.10) | 0.00 (-0.03; 0.03) | 0.00 (-0.04; 0.05) | 0.04 (-0.00; 0.09) | 0.06 (0.01; 0.10) |
| Conditional relative weight | | | | | | | | | | | |
| CWh 0-1 y | -0.04 (-0.12; 0.04) | 0.07 (-0.01; 0.16) | 0.03 (-0.05; 0.12) | -0.01 (-0.10; 0.09) | -0.04 (-0.13; 0.05) | -0.01 (-0.11; 0.10) | 0.01 (-0.03; 0.06) | 0.06 (-0.00; 0.13) | 0.03 (-0.06; 0.12) | 0.25 (0.15; 0.35) | 0.21 (0.12; 0.30) |
| CWh 1-4 y | 0.03 (-0.06; 0.11) | -0.05 (-0.14; 0.04) | 0.08 (-0.01; 0.18) | -0.02 (-0.12; 0.08) | -0.02 (-0.11; 0.08) | -0.03 (-0.14; 0.07) | 0.03 (-0.03; 0.08) | 0.12 (0.05; 0.18) | 0.12 (0.03; 0.23) | 0.56 (0.47; 0.65) | 0.45 (0.37; 0.54) |
| CWh 4-11 y | -0.01 (-0.09; 0.07) | 0.10 (0.02; 0.18) | 0.12 (0.03; 0.20) | -0.04 (-0.13; 0.06) | -0.15 (-0.24; -0.07) | 0.03 (-0.07; 0.13) | 0.00 (-0.02; 0.02) | 0.10 (0.04; 0.17) | 0.13 (0.04; 0.22) | 0.62 (0.55; 0.68) | 0.53 (0.47; 0.59) |
| CWh 11-15 y | 0.08 (0.02; 0.15) | 0.04 (-0.04; 0.12) | 0.14 (0.06; 0.24) | 0.00 (-0.09; 0.01) | -0.18 (-0.27; -0.10) | 0.05 (-0.05; 0.15) | 0.03 (0.01; 0.05) | 0.15 (0.09; 0.21) | 0.11 (0.03; 0.20) | 0.45 (0.40; 0.50) | 0.39 (0.34; 0.46) |
| CWh 15-18 y | 0.05 (-0.01; 0.12) | -0.02 (-0.11; 0.06) | 0.21 (0.12; 0.30) | 0.14 (0.04; 0.24) | -0.10 (-0.19; -0.02) | 0.20 (0.09; 0.29) | 0.03 (0.01; 0.05) | 0.16 (0.10; 0.22) | 0.13 (0.04; 0.22) | 0.51 (0.50; 0.53) | 0.47 (0.43; 0.50) |
| Conditional length/height | | | | | | | | | | | |
| CH 0-1 y | 0.01 (-0.07; 0.09) | -0.04 (-0.13; 0.03) | 0.02 (-0.07; 0.13) | 0.08 (-0.01; 0.17) | 0.17 (0.08; 0.26) | 0.03 (-0.07; 0.12) | -0.02 (-0.07; 0.02) | 0.09 (0.02; 0.15) | 0.01 (-0.07; 0.10) | 0.01 (-0.08; 0.11) | 0.08 (-0.00; 0.17) |
| CH 1-4 y | -0.03 (-0.12; 0.05) | 0.16 (0.08; 0.25) | 0.13 (0.03; 0.22) | 0.06 (-0.04; 0.16) | 0.11 (0.02; 0.21) | 0.01 (-0.09; 0.12) | 0.03 (-0.02; 0.08) | 0.10 (0.03; 0.17) | 0.11 (0.01; 0.20) | 0.12 (0.01; 0.22) | 0.22 (0.12; 0.31) |
| CH 4-11 y | -0.06 (-0.13; 0.02) | -0.10 (-0.17; -0.02) | -0.02 (-0.11; 0.07) | 0.05 (-0.04; 0.15) | 0.04 (-0.04; 0.13) | 0.04 (-0.06; 0.14) | -0.00 (-0.02; 0.02) | 0.08 (0.02; 0.14) | 0.20 (0.11; 0.29) | 0.12 (0.04; 0.20) | 0.12 (0.04; 0.20) |
| CH 11-15 y | 0.03 (-0.04; 0.09) | -0.07 (-0.15; 0.01) | -0.01 (-0.10; 0.08) | -0.02 (-0.11; 0.08) | -0.07 (-0.15; 0.02) | 0.02 (-0.09; 0.12) | -0.01 (-0.03; 0.02) | 0.08 (0.02; 0.15) | 0.02 (-0.07; 0.10) | -0.01 (-0.07; 0.06) | 0.08 (0.01; 0.13) |
| CH 15-18 y | 0.04 (-0.02; 0.11) | 0.05 (-0.03; 0.13) | -0.10 (-0.18; -0.01) | 0.00 (-0.09; 0.10) | -0.05 (-0.14; 0.03) | 0.01 (-0.09; 0.11) | 0.01 (-0.01; 0.03) | -0.00 (-0.07; 0.06) | 0.00 (-0.08; 0.09) | -0.05 (-0.07; -0.03) | -0.01 (-0.05; 0.05) |

HbA1c: glycated hemoglobin, CRP: reactive-C protein, TC: total cholesterol, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TGL: triglycerides, DBP: diastolic blood pressure, SBP: systolic blood pressure, BMI: body mass index, CC: waist circumference, CWh: conditional relative weight gain, CH : conditional height gain

Data are β (95% CI). The outcome variables were normalized. Regression coefficient (β) values were calculated with linear regression models and indicate the SD change in the outcome per SD change in the predictor. All models were adjusted for mother's education (years of schooling), household wealth (in minimum wages) at birth and skin color of the adolescent. The models for plasma glucose concentrations were further adjusted for time from previous meal.