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DISSERTATION

Geburtsgewicht und späteres Übergewichtsrisiko – epidemiologische und tierexperimentelle Untersuchungen

zur Erlangung des akademischen Grades
Doctor rerum medicinalium (Dr. rer. medic.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

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Datum der Promotion: 05.12.2014

Inhaltsverzeichnis

Abkürzungsverzeichnis

AGA	appropriate-for-gestational-age
AGA-in-NW	appropriate-for-gestational-age Tiere, aufgezogen in normalen Würfen
AGRP	Agouti-related Peptide
ARC	Nucleus arcuatus hypothalami
AUCG	area under the curve of glucose
BMI	Body-Mass-Index
cDNA	complementary DNA
CI	Konfidenzintervall
DNA	Desoxyribonukleinsäure
EZ	Einzelzellpool
g	Gramm
GAL	Galanin
GGew	Geburtsgewicht
GTT	Glukosetoleranztest
HE/HF	high-energy/high-fat
IRI/BG	Insulin/Blutglukose-Ratio
kcal	Kilokalorien
kg	Kilogramm
KW	kleiner Wurf
LBW	low birth weight
LMD	Lasermikrodissektion
LT	Lebenstag
mRNA	messenger-Ribonukleinsäure
MW	Mittelwert
NPY	Neuropeptid Y
n.s.	nicht signifikant
NW	normaler Wurf
OR	Odds Ratio
PCR	Polymerasekettenreaktion
POMC	Proopiomelanocortin
RNA	Ribonukleinsäure
SD	Standardabweichung
SEM	Standardfehler des Mittelwertes
SGA	small-for-gestational-age
SGA-in-NW	small-for-gestational-age Tiere, aufgezogen in normalen Würfen
SGA-in-KW	small-for-gestational-age Tiere, aufgezogen in kleinen Würfen
WHR	waist-to-hip-ratio
µm	Mikrometer

1. Zusammenfassung

1.1 Abstract

1.1.1 Abstract - englisch

Since the 1990s, epidemiological data have shown that low birth weight (LBW) is associated with increased risk for long-term health adversity, especially concerning cardiovascular diseases, risk of type 2 diabetes and the metabolic syndrome, in which overweight is of central pathogenetic importance. Causal mechanisms, however, of the so-called 'small-baby-syndrome' are still unclear. On the other hand, a number of studies have reported that individuals born with high birth weight, induced by prenatal overnutrition, are also at increased risk for type 2 diabetes later in life. In the pathogenesis of the 'small-baby-syndrome', early postnatal overnutrition is attracting increasing attention as a possible link between low birth weight and later 'diabesogenic' risk. Thus, the objective of this translational work was first, to investigate the relation between birth weight and later overweight risk with an epidemiologically approach and second, to examine the influence of early postnatal overnutrition in low birth weight animals experimentally.

A comprehensive meta-analysis, which included 66 studies involving more than 640,000 persons from 26 countries globally, aged 1-75 years, revealed a linear positive relationship between birth weight and later, long-term overweight risk over nearly the entire birth weight spectrum. Individuals with a birth weight > 4000 g showed an almost doubled long-term overweight risk as compared with normal birth weight (2500-4000 g) subjects. In contrast, low birth weight was followed by a decreased overweight risk in later life.

In a 'genuine' animal model, newly established here, rats with LBW were followed up into late adult age, and developed increased risk for diabetogenic alterations and hyperphagia only if they were exposed to neonatal overfeeding. Using the highly specific and cutting edge technology of lasercapture microdissection (LMD)-based neuropeptide expression analyses in single neuron pools of the arcuate hypothalamic nucleus (ARC) revealed a nearly unchanged gene expression of orexigenic neuropeptides despite marked hyperleptinemia and hyperinsulinemia. Gene expression of the anorexigenic neurohormone proopiomelanocortin (POMC) was significantly decreased, even when referred to the regulating satiety signals, leptin and insulin, from the periphery. This strongly indicates a neonatally acquired malprogramming of the anorexigenic POMC-system due to neonatal overfeeding in LBW rats.

Together our epidemiological and experimental results show that high birth weight is an independent risk factor for long-term overweight risk while this could not be confirmed for low birth weight. However, neonatal overfeeding in rats with low birth weight plays a crucial role in the development of 'diabesogenic' alterations in later life. In conclusion, both the prenatal as well as neonatal period seem to be critical phases for the determination of long-term adverse health outcomes in terms of the metabolic syndrome and, consequently, may potentially allow measures of primary prevention.

1.1.2 Abstract - deutsch

Seit Mitte der 1990er Jahre wurde in zahlreichen epidemiologischen Studien ein Zusammenhang zwischen einem verminderten Geburtsgewicht (GGew) und der Entwicklung von kardiovaskulären Erkrankungen, Typ 2 Diabetes und Symptomen des metabolischen Syndroms im späteren Leben beobachtet (’small-baby-syndrome’), in dem Übergewicht eine zentrale pathophysiologische Rolle spielt. Zugrundeliegende ätiopathogenetische Mechanismen sind bislang ungeklärt. Andererseits existieren Studien, in denen beobachtet wurde, dass ein zu hohes GGew, verursacht durch pränatale Überernährung, ebenfalls einen Risikofaktor für spätere Erkrankungen, wie z.B. Typ 2 Diabetes, darstellt. In der Pathogenese des ’small-baby-syndrome’ rückt als möglicher ’link’ zwischen einem verminderten GGew und dem späteren Erkrankungssrisiko eine frühpostnatale Überernährung zunehmend in den Fokus. Ziel der vorliegenden Arbeit war es daher, in einem translationalen Ansatz den Zusammenhang zwischen dem GGew und dem späteren Übergewichtsrisiko epidemiologisch zu untersuchen und in einer tierexperimentellen Langzeitstudie der Frage nachzugehen, inwieweit eine frühpostnatale Überernährung nach zu niedrigem GGew das spätere Übergewichts- und Diabetesrisiko beeinflusst.

Im Rahmen einer Metaanalyse, die 66 Studien mit mehr als 640.000 Personen im Alter von ein bis 75 Jahren aus 26 Ländern weltweit berücksichtigte, zeigte sich über das gesamte Geburtsgewichtsspektrum ein positiver Zusammenhang zwischen dem GGew und dem späteren Übergewichtsrisiko. Dabei hatten Kinder mit einem GGew über 4000 g ein fast doppelt so hohes Risiko im späteren Leben übergewichtig zu werden wie normalgewichtige Neugeborene. Dagegen ging ein niedriges GGew mit einem signifikant verminderten Risiko für Übergewicht im späteren Leben einher.

In einem hier neu etablierten, ’genuinen’ Tiermodell für das ’small-baby-syndrome’ kam es bei adulten Ratten mit einem verminderten GGew nur dann zu einer Disposition für diabetogene Stoffwechselstörungen und Hyperphagie, wenn sie einer neonatalen Überernährung ausgesetzt waren. Genexpressionsanalysen in Einzelneuronen des Nucleus arcuatus hypothalami (ARC), die mit Hilfe einer hier ebenfalls neu etablierten Methodenkombination aus Lasermikrodissektion und quantitativer real-time PCR durchgeführt wurden, ergaben eine nahezu unveränderte Expression orexigener Neuropeptide im Vergleich zu den Kontrolltieren, trotz ausgeprägter Hyperleptinämie und Hyperinsulinämie. Die Expression des anorexigenen Proopiomelanocortins (POMC) war dagegen, sogar unter Bezug auf die regulierenden peripheren Sättigungssignale Leptin und Insulin, signifikant vermindert, was für eine neonatal erworbene Fehlprogrammierung des anorexigenen POMC-Systems infolge frühpostnataler Überernährung spricht.

Zusammenfassend zeigen diese Untersuchungen, dass ein zu hohes GGew einen unabhängigen Risikofaktor für späteres Übergewicht darstellt, während es keine Hinweise darauf gibt, dass dies auch nach vermindertem GGew der Fall ist, wie in der Hypothese des ’small-baby-syndrome’ ursprünglich postuliert. Allerdings scheint, auch bei Kindern mit einem verminderten GGew, neonatale Überernährung einen Risikofaktor für die Entwicklung von Übergewicht und diabetogenen Stoffwechselstörungen im späteren Leben darzustellen. Somit erweisen sich sowohl die Prä- als auch die Neonatalphase als kritische Zeitfenster, in denen das Risiko für spätere Krankheiten im Sinne des metabolischen Syndroms geprägt wird und folglich einer primärpräventiven Intervention zugänglich sein dürfte.

1.2 Einleitung und Zielstellung

Mit einer für das Jahr 2030 vorhergesagten Prävalenz von 58% in der erwachsenen Weltbevölkerung gehören Übergewicht und Adipositas schon heutzutage zu den größten Gesundheitsproblemen weltweit [Kelly *et al.* 2008]. Bedingt durch ihre konsekutiven diabetischen und kardiovaskulären Erkrankungen stellen sie eine immense medizinische wie auch gesundheitspolitische Herausforderung dar [Haslam und James 2005, Hossain *et al.* 2007, Knoll und Hauner 2008, Scully 2012]. Um die geradezu epidemieartige Ausbreitung einzudämmen, ist es dringend erforderlich, frühzeitig erfassbare Risikofaktoren aufzudecken, um damit das Augenmerk zunehmend auf Maßnahmen einer Primärprävention richten zu können.

In den frühen neunziger Jahren wiesen die Arbeitsgruppen um Hales und Barker erstmals auf einen Zusammenhang zwischen vermindertem Geburtsgewicht und einem erhöhten Risiko für spätere kardiovaskuläre Erkrankungen, Typ 2 Diabetes und die Entwicklung von Symptomen des metabolischen Syndroms hin [Barker *et al.* 1989 und 1993, Hales und Barker 1992]. Sie postulierten, dass es sich bei diesem, auch als 'small-baby-syndrome' bekannt gewordenen Zusammenhang, vor allem um die Folgen einer pränatalen Unterernährung handelt [Hales und Barker 1992 und 2001]. Die zugrundeliegenden pathophysiologischen Mechanismen sind bis heute ungeklärt. Im Zusammenhang mit dem 'small-baby-syndrome' ist bisher ebenso unklar, ob demzufolge auch eine inverse Beziehung zwischen dem Geburtsgewicht und dem späteren Risiko für die Entwicklung von Übergewicht besteht, welches im Symptomenkomplex des metabolischen Syndroms den entscheidenden pathophysiologischen Risikofaktor darstellt [Cameron *et al.* 2008, Eckel *et al.* 2010]. Bisher existierte in der internationalen Literatur keine umfassende Datensynthese zu dieser Fragestellung.

Demgegenüber stehen Ergebnisse einer Reihe von epidemiologischen Studien, die berichten, dass nicht nur ein geringes, sondern auch ein hohes Geburtsgewicht infolge pränataler Überernährung einen Risikofaktor für die spätere Entwicklung von Krankheiten darstellt. So konnten Metaanalysen zeigen, dass sowohl zu niedriges als auch zu hohes Geburtsgewicht mit einem erhöhten Risiko für Typ 2 Diabetes und Hypertonie im späteren Leben assoziiert sind [Gamborg *et al.* 2007, Harder *et al.* 2007a, Huxley *et al.* 2002].

Für den Effekt des verminderten Geburtsgewichtes auf spätere Krankheitsrisiken wird seit langem von unserer Arbeitsgruppe, wie auch zunehmend international, einer gesteigerten neonatalen Gewichtszunahme im Sinne eines 'rapid neonatal weight gain' infolge neonataler Überernährung maßgebliche Bedeutung beigemessen [Dörner und Plagemann 1994, Eriksson *et al.* 1999, *Schellong et al.* 2008, Singhal *et al.* 2007]. Tierexperimentelle Untersuchungen unserer Arbeitsgruppe zeigen, dass ein fetaler und/oder neonataler Hyperinsulinismus infolge einer fetalen und/oder neonatalen Überernährung zu einer dauerhaften hypothalamischen Fehlregulation von Neuropeptiden führen kann, welche an der zentralnervösen Regulation von Nahrungsaufnahme, Stoffwechsel und Körpergewicht maßgeblich beteiligt sind. Die Folge ist

eine dauerhaft erhöhte Disposition für Übergewicht, Diabetes und konsekutive Erkrankungen [Plagemann 2008, Plagemann *et al.* 1999a, 1999b, 2009 und 2012b, *Schellong et al.* 2008 und 2009]. Der mögliche Einfluss einer neonatalen Überernährung bei Tieren mit einem 'genuin' verminderten Geburtsgewicht auf das spätere Übergewichts- und Diabetesrisiko wurde tierexperimentell im Langzeitversuch bislang nicht untersucht.

Nahrungsaufnahme und Körpergewicht werden vornehmlich im Nucleus arcuatus hypothalami (ARC), einem Kerngebiet des Hypothalamus, durch orexigene, also die Nahrungsaufnahme stimulierende (z.B. Neuropeptid Y, NPY) bzw. anorexigene, die Nahrungsaufnahme hemmende (z.B. Proopiomelanocortin, POMC) Neuropeptide reguliert. Während in der internationalen Literatur Konsens darüber besteht, dass es unter Nahrungskarenz im Tiermodell der Ratte zu einer hypothalamischen Up-Regulation orexigener Neuropeptide kommt [Ahima *et al.* 1999, Bi *et al.* 2003, Dallman *et al.* 1999, Palou *et al.* 2009, Pritchard *et al.* 2003], ist die Befundlage bezüglich der Regulation des entscheidenden anorexigenen Neuropeptids POMC im Fastenzustand bislang uneindeutig [Ahima *et al.* 1999, Bi *et al.* 2003, Dallman *et al.* 1999, Palou *et al.* 2009, Pritchard *et al.* 2003]. Ursachen dafür könnten in der neuromorphologischen Spezifität des Untersuchungsmaterials und/oder in der Sensitivität der angewandten Methodik liegen. Um jedoch eindeutige Aussagen hinsichtlich neuropeptiderger Regelmechanismen im Rahmen der Hunger- und Sättigungsregulation, gerade auch innerhalb hochsensibler Thematiken, wie bspw. der des 'small-baby-syndroms', treffen zu können, ist die Verwendung hochsensitiver und gleichzeitig hochspezifischer Untersuchungsmethoden eine Grundvoraussetzung.

Für die vorliegende Promotionsarbeit ergaben sich somit folgende Zielstellungen:

1. Epidemiologische Untersuchungen zum Zusammenhang zwischen dem Geburtsgewicht und dem späteren Übergewichtsrisiko.

Im epidemiologischen Teil dieser Promotionsarbeit sollte in einem systematischen Review und einer Metaanalyse untersucht werden, ob der laut 'small-baby-syndrome'-Hypothese postulierte inverse Zusammenhang zwischen dem Geburtsgewicht und dem Risiko für die Entwicklung von Übergewicht im späteren Leben tatsächlich besteht [*Schellong et al.* 2012].

2. Methodenetablierung zur Lasermikrodissektion und Neuropeptidexpression.

In Vorbereitung einer tierexperimentellen Langzeitstudie zum 'small-baby-syndrome' sollte zunächst eine Methodenkombination aus Lasermikrodissektion (LMD) und quantitativer real-time PCR etabliert werden, um hochsensitive Genexpressionsanalysen in neuromorphologisch spezifischem Probenmaterial durchführen zu können. Hierzu sollten im Tiermodell der Ratte die Auswirkungen einer Nahrungskarenz und anschließender peripherer Glukosegabe auf die Genexpression orexigener und anorexigener Neuropeptide im ARC untersucht werden [Landmann, *Schellong et al.* 2012].

3. Tierexperimentelle Langzeitstudie zum 'small-baby-syndrome'.

In einem tierexperimentellen Langzeitversuch sollte mit Hilfe eines neu etablierten, 'genuinen' Tiermodells für das 'small-baby-syndrome' untersucht werden, inwieweit bei Ratten mit einem verminderten Geburtsgewicht eine vermehrte neonatale Gewichtszunahme infolge neonataler Überernährung einen Einfluss auf das spätere Übergewichts- und Diabetesrisiko hat. Dabei sollte auch die Futteraufnahme der Tiere im adulten Alter untersucht werden. Auf molekularbiologischer Ebene sollten mittels der zuvor etablierten LMD-basierten Genexpressionsanalyse in Einzelneuronen des ARC Neuropeptid-Expressionsmuster charakterisiert werden, die in die hypothalamische Regulation von Nahrungsaufnahme, Körpergewicht und Stoffwechsel entscheidend involviert sind, um mögliche Pathomechanismen aufzudecken, die dem 'small-baby-syndrome' zugrunde liegen könnten [*Schellong et al.* 2013].

1.3 Methodik

1.3.1 Epidemiologische Untersuchungen

Mit Hilfe eines systematischen Reviews und einer Metaanalyse der bis *dato* publizierten Literatur untersuchten wir, ob tatsächlich ein Zusammenhang zwischen dem Geburtsgewicht und dem Risiko für die Entwicklung von Übergewicht im späteren Leben besteht [*Schellong et al.* 2012]. Die Durchführung des systematischen Reviews und der Metaanalyse erfolgte in Übereinstimmung mit den Vorgaben des PRISMA Statement (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) für systematische Übersichten und Metaanalysen [Moher *et al.* 2009]. In Erweiterung eines früheren systematischen Reviews unserer Arbeitsgruppe [Harder *et al.* 2007b] wurde eine umfangreiche Literaturrecherche in den Datenbanken MEDLINE und Embase (1966 - Januar 2011) mit den Suchbegriffen "birth weight", "overweight", "obesity" und "adiposity" durchgeführt, um alle relevanten Studien zu erfassen, die den o.g. Zusammenhang untersucht haben. Eingeschlossen wurden alle als Originalarbeit publizierten Studien, in denen bei den Studienteilnehmern Angaben zum Geburtsgewicht in mindestens zwei Kategorien gemacht wurden, oder Studien, in denen eine Odds Ratio (OR) mit 95% Konfidenzintervall (95% CI) für Übergewicht pro Einheit Geburtsgewicht berichtet wurde und die mindestens eine Angabe zum Anteil übergewichtiger Probanden in mindestens einem Lebensalter enthielten.

Aus den extrahierten Daten wurden zunächst mit einem dichotomen Ansatz gepoolte Effektschätzer berechnet. Dabei wurde eine gepoolte Odds Ratio und das dazugehörige 95% CI für Übergewicht nach niedrigem Geburtsgewicht (< 2500 g) im Vergleich zum darüber liegenden Geburtsgewicht (> 2500 g) bzw. im Vergleich zu einem normalen Geburtsgewicht (2500 bis 4000 g = Referenzkategorie) berechnet. Anschließend wurde dieses Verfahren für hohes Geburtsgewicht (> 4000 g) wiederholt.

Um eine etwaige Heterogenität zwischen den Studienergebnissen zu beurteilen, wurde der Cochrane-Q-Test genutzt. Der Grad der Heterogenität wurde nach Higgins als I^2 bestimmt [Higgins *et al.* 2003]. Bei Vorliegen von Heterogenität erfolgte die Berechnung der gepoolten Effektschätzer aus den Effektschätzern der Einzelstudien mit dem 'random effects model', welches die Variabilität zwischen den Studien berücksichtigt und in der Regel zu einer konservativeren Schätzung führt. Die Ergebnisse wurden in Form von 'forest plots' graphisch dargestellt. Um mögliche Ursachen für Heterogenität zu identifizieren und um die Stabilität der gepoolten Schätzer zu überprüfen, wurden Sensitivitätsanalysen durchgeführt. Für bestimmte Merkmale, wie z.B. Studiendesign oder Klassifikationskriterien für Übergewicht, wurden gesonderte Analysen vorgenommen und separate Odds Ratios berechnet. Das mögliche Vorliegen von 'publication bias' wurde zunächst graphisch mit Hilfe eines 'funnel plots' geprüft, in dem die Effektschätzer der einzelnen Studien gegen deren Standardfehler aufgetragen wurden. Die formale Überprüfung einer 'funnel plot' Asymmetrie erfolgte mittels linearer Regressionsanalyse mit dem Egger Test [Egger *et al.* 1997] und mit dem Rangkorrelationstest nach Begg [Begg und Mazumdar 1994].

Zur Beschreibung der Form des kontinuierlichen Zusammenhanges zwischen dem Geburtsgewicht und dem späteren Übergewichtsrisiko verwendeten wir die Methode der Metaregression [Berlin *et al.* 1993].

Abschließend führten wir eine separate Analyse mit denjenigen Studien durch, die confounder-adjustierte Schätzer berichtet haben, um zu überprüfen, ob die Stärke des beobachteten Zusammenhanges durch Störgrößen (confounder) beeinflusst wird.

1.3.2 Methodenetablierung zur Lasermikrodissektion und Neuropeptidexpression

In Vorbereitung einer tierexperimentellen Langzeitstudie zum 'small-baby-syndrome' sollte zunächst eine molekularbiologische Methodenkombination etabliert werden, welche in neuromorphologisch spezifischem Probenmaterial aus histologischen Gehirnschnitten eine präzise Genexpressionsanalyse von Neuropeptiden erlaubt, die in die Hunger- und Sättigungsregulation involviert sind. Deshalb erfolgte die Etablierung der Methode der LMD zur Präparation von Einzelneuronen aus dem ARC und anschließender mRNA-Expressionsanalyse mittels quantitativer real-time PCR unter definierten Experimentalbedingungen am Tiermodell der Ratte, indem adulte Tiere einer Nahrungskarenz ausgesetzt wurden [Landmann, *Schellong et al.* 2012]. Hierzu wurde eine Versuchsgruppe *ad libitum* gefüttert, während eine zweite Gruppe einer 12-stündigen Nahrungskarenz ausgesetzt wurde. Einer dritten Gruppe wurde nach einer ebenfalls 12-stündigen Nahrungskarenz eine 20%ige Glukoselösung (1,5 g Glukose/kg Körpergewicht) intraperitoneal appliziert. Dreißig Minuten nach Glukoseapplikation erfolgte die Tötung aller Tiere durch rasche Dekapitation. Aus dem gewonnenen Trunkalblut wurden Blutglukose (Glukoseoxidase-Peroxidase-Methode, Dr. Lange GmbH), Plasmainsulin (Insulin

Radioimmunoassay (RIA) Kit, Adaltis) und Plasmaleptin (Rattenleptin RIA Kit, Linco) bestimmt. Die Gehirne wurden zügig entnommen und mit Hilfe von tiefgekühltem Isopentan eingefroren. In einem Kryostat wurden im Bereich des Hypothalamus 10 µm dicke koronare Gefrierschnitte in Serie gefertigt, mittels Kresylviolett übersichtsgefärbt und anschließend der LMD zugeführt. Über die gesamte rostro-kaudale Ausdehnung des ARC, entsprechend den Koordinaten im Rattengehirn-Atlas nach Paxinos und Watson [Paxinos und Watson 1986], wurden unter Verwendung des AS/LMD-Gerätes von Leica (Leica Microsystems CMS GmbH) Proben gewonnen. Dabei wurde in folgender Weise verfahren: Auf der linken Seite des Hypothalamus wurde der ARC als zusammenhängender Zellverband isoliert. Anschließend wurden im selben Schnitt von der kontralateralen Seite des ARC Einzelneuronen isoliert und zu insgesamt 100 Neuronen je Probe (Tier) gepoolt.

Aus den isolierten Einzelneuronen bzw. Zellverbänden des ARC wurde anschließend mit kommerziellen Kits die RNA isoliert, in cDNA umgeschrieben und anschließend in der quantitativen real-time PCR (Applied Biosystems 7500) amplifiziert. Die Analyse der Neuropeptide Agouti-related Peptide (AGRP) und POMC, mit beta Actin als Referenzgen, erfolgte als Duplex-PCR in Triplikaten [Plagemann *et al.* 2010]. Es wurden ausschließlich kommerzielle, für das Zielgen spezifische, intronüberspannende Primer und Sonden der Firma Applied Biosystems verwendet, um eine Vervielfältigung genomischer DNA zu vermeiden. Die Berechnung der relativen Genexpression erfolgte mittels relativer Quantifizierung in Anlehnung an die $2^{-\Delta Ct}$-Methode unter Berücksichtigung der jeweiligen PCR-Effizienzen und durch Normalisierung auf das Referenzgen beta Actin [Schmittgen und Livak 2008].

1.3.3 Tierexperimentelle Langzeitstudie

Um die Auswirkungen einer neonatalen Überernährung bei Ratten mit einem verminderten Geburtsgewicht im Langzeitversuch zu untersuchen, etablierten wir ein neues, 'genuines' Tiermodell für das 'small-baby-syndrome' [Neitzke *et al.* 2011, *Schellong et al.* 2013]. In Anlehnung an klinische Kriterien definierten wir neugeborene Ratten normaler Rattenmütter als 'small-for-gestational-age' (SGA), wenn ihr Geburtsgewicht unterhalb der Untergrenze des 95% CI des mittleren Geburtsgewichts gleichgeschlechtlicher Tiere des zugehörigen Wurfes lag. Tiere, deren Geburtsgewicht innerhalb der Grenzen des 95% CI des Geburtsgewichts gleichgeschlechtlicher Tiere des zugehörigen Wurfes lag, wurden für 'appropriate-for-gestational-age' (AGA) erklärt. So definierte Tiere wurden bis zum 21. Lebenstag (LT) durch Aufzucht in normalen Würfen ('Nestern') mit 12 Nachkommen pro Mutter normal ernährt (SGA-in-NW) oder in kleinen Würfen ('Nestern') mit nur 3 Tieren überernährt (SGA-in-KW). Als Kontrolltiere galten bei Geburt normalgewichtige Tiere in normalen Würfen (AGA-in-NW). Nach dem Absetzen von der Mutter (21. LT) fand die Aufzucht aller Tiere in randomisierter Gruppenhaltung bis in das hohe adulte Alter statt. Es wurden in regelmäßigen Abständen Körpergewicht und Körperlänge

bestimmt und das relative Körpergewicht berechnet. Nach Tötung der Tiere am 560. LT erfolgte die Bestimmung des Körperfettanteils mit Hilfe des Soxhlet-Verfahrens [Plagemann *et al.* 2009].

1.3.3.1 Metabolische Parameter

Aus dem Nüchternblut bzw. aus dem bei Dekapitation gewonnenen Trunkalblut wurden Blutglukose (Glukoseoxidase-Peroxidase-Methode, Dr. Lange GmbH), Plasmainsulin (Insulin RIA Kit, Adaltis) und Plasmaleptin (Rattenleptin RIA Kit, Linco) bestimmt. Die Insulin/Blutglukose-Ratio (IRI/BG-Ratio) wurde berechnet, um Hinweise auf eine mögliche Insulinresistenz zu erhalten [Griffin *et al.* 1998, Harder *et al.* 2001].

Für die Durchführung von Glukosetoleranztests (GTT) am 130. und 530. LT wurden die Tiere zunächst einer 16-stündigen Nahrungskarenz ausgesetzt. Nach Nüchternblutentnahme und intraperitonealer Applikation einer 20%igen Glukoselösung (1,5 g Glukose/kg Körpergewicht) wurde nach 15, 30 und 90 Minuten die Blutglukose bestimmt und anschließend die Fläche unter der Glukosekurve (engl. area under the curve of glucose, AUCG) berechnet.

1.3.3.2 Nahrungsaufnahme

Im adulten Alter von 470 bis 560 Lebenstagen wurde die Futteraufnahme der Tiere erfasst. Dazu wurde zunächst über einen Zeitraum von 30 Tagen die tägliche Futteraufnahme einer Standarddiät (ssniff, V1534-0; umsetzbare Energie: 3,1 kcal/g) bestimmt und anschließend über einen Zeitraum von 60 Tagen die tägliche Aufnahme einer hyperkalorischen Hochfettdiät (high-energy/high-fat (HE/HF)-Diät; Sonderdiät, Altromin 132006; umsetzbare Energie: 4,1 kcal/g). Die Tiere erhielten Futter und Trinkwasser *ad libitum*.

1.3.3.3 Hypothalamische Neuropeptidexpression

Bei Tötung am 560. LT wurden die Gehirne von Versuchs- und Kontrolltieren zügig entnommen und mit Hilfe von tiefgekühltem Isopentan eingefroren. In einem Kryostat wurden im Bereich des Hypothalamus 10 µm dicke koronare Gefrierschnitte in Serie gefertigt, mittels Kresylviolett übersichtsgefärbt und anschließend der zuvor etablierten LMD zugeführt (siehe 1.3.2). Über die gesamte rostro-kaudale Ausdehnung des ARC wurden in zufälliger Reihenfolge Einzelneuronen isoliert und zu insgesamt 100 Neuronen je Probe (Tier) gepoolt.

Mit Hilfe kommerzieller Kits wurde aus den isolierten Einzelneuronen des ARC die RNA isoliert, in cDNA umgeschrieben und anschließend mittels quantitativer real-time PCR amplifiziert. Die Analyse der Neuropeptide NPY, AGRP, Galanin (GAL) und POMC, mit beta Actin als Referenzgen, erfolgte als Duplex-PCR in Triplikaten [Plagemann *et al.* 2010]. Zur Durchführung und Auswertung der Genexpressionsanalysen siehe Kapitel 1.3.2.

1.3.4 Statistik

Alle Analysen im Rahmen der Metaanalyse erfolgten unter Verwendung des Softwarepaketes STATA 11.0 (Stata Corp., College Station, TX, USA).

Aus den Daten der tierexperimentellen Untersuchungen wurden Gruppen-Mittelwerte (MW), die dazugehörige Standardabweichung (SD) bzw. der Standardfehler des Mittelwertes (SEM) berechnet. Mittelwertvergleiche zwischen zwei Gruppen erfolgten mittels U-Test nach Mann und Whitney. Der Vergleich der Daten von mehr als zwei Gruppen erfolgte mit der einfachen Varianzanalyse (Analysis of Variance, ANOVA) und anschließendem Tukey's HSD post-hoc-Test. Zusammenhänge zwischen Variablen wurden mit dem Spearman-Rangkorrelationstest untersucht. Das Signifikanzniveau wurde auf $p<0,05$ festgelegt. Alle statistischen Analysen wurden mit SPSS (Version 19.0) bzw. GraphPad Prism (Version 4.03) durchgeführt.

1.4 Ergebnisse

Im Folgenden sind die Kernbefunde der dieser Promotionsarbeit zugrundeliegenden drei Publikationen umrissen (siehe Kapitel 4 'Druckexemplare'). Auf Originalabbildungen der jeweiligen Publikationen wird an entsprechender Stelle verwiesen.

1.4.1 Epidemiologische Untersuchungen zum Zusammenhang zwischen Geburtsgewicht und späterem Übergewichtsrisiko

Von insgesamt 3.513 potentiell für unsere Fragestellung relevanten Studien gingen 108 Studien in den systematischen Review ein, die 1.485.561 Studienteilnehmer im Alter von 6 Monaten bis 79 Jahren umfassten. 94 der 108 Studien (87%) beschrieben einen positiven Zusammenhang zwischen dem Geburtsgewicht und dem späteren Übergewichtsrisiko. 6 Studien (5,6%) beobachteten einen U-förmigen Zusammenhang, d.h., dass sowohl ein geringes als auch ein hohes Geburtsgewicht einen Risikofaktor für späteres Übergewicht darstellten. In 7 Studien (6,5%) konnte kein Zusammenhang zwischen dem Geburtsgewicht und dem späteren Risiko für Übergewicht beobachtet werden. Nur eine Studie (0,9%) berichtete einen inversen Zusammenhang zwischen dem Geburtsgewicht und dem späteren Übergewichtsrisiko (**Abb. 1**).

Von den Studien des systematischen Reviews konnten aus methodischen Gründen 42 Studien nicht in die Metaanalyse eingeschlossen werden, weil bspw. die genaue Definition der Geburtsgewichtsperzentilen fehlte oder die in den Studien berichteten Daten für eine quantitative Analyse nicht ausreichend waren. Somit gingen insgesamt 66 Studien in die Metaanalyse ein, 58 Kohortenstudien und 8 Fall-Kontroll-Studien, die 643.902 Studienteilnehmer auf 6 Kontinenten im Alter von 1-75 Jahren umfassten. Hiervon berichteten 59 Studien (89,4%) einen positiven Zusammenhang zwischen dem Geburtsgewicht und dem späteren Übergewichtsrisiko. 3 Studien (4,5%) zeigten einen U-förmigen Zusammenhang. In 4 Studien (6,1%) konnte kein Zusammenhang nachgewiesen werden. In keiner der Studien wurde ein inverser Zusammenhang zwischen dem Geburtsgewicht und dem späteren Übergewichtsrisiko beschrieben.

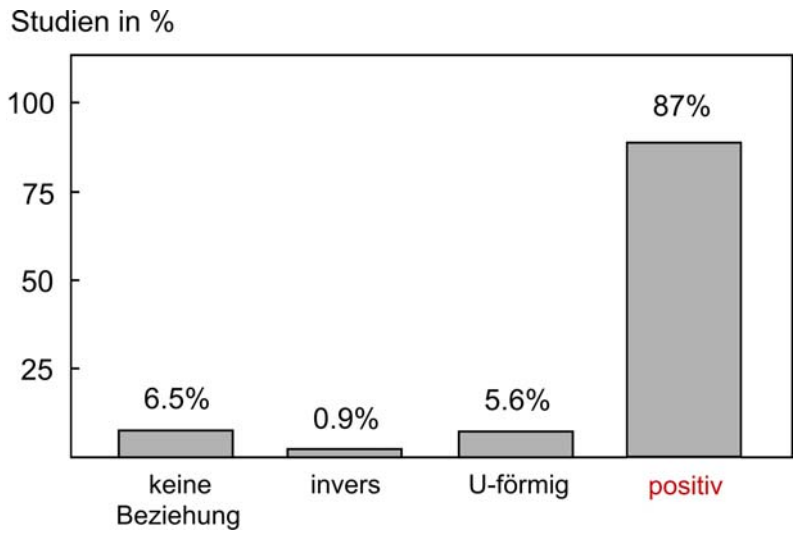

Abb. 1: Zusammenhang zwischen Geburtsgewicht und späterem Übergewichtsrisiko. Systematischer Review der publizierten Literatur (1966 – Januar 2011) zum Zusammenhang zwischen dem Geburtsgewicht und dem späterem Übergewichtsrisiko [*Schellong et al.* 2012]. Angabe der Studienanzahl für den jeweiligen Zusammenhang in Prozent, basierend auf 108 Studien aus 30 Ländern auf 6 Kontinenten.

Der dichotome Vergleich ergab, dass ein niedriges Geburtsgewicht (< 2500 g), verglichen mit einem Geburtsgewicht > 2500 g, mit einem verminderten Risiko für späteres Übergewicht assoziiert ist (OR=0,67; 95% CI: 0,59-0,76), ebenso wie ein niedriges Geburtsgewicht (< 2500 g), verglichen mit einem normalen Geburtsgewicht (2500-4000 g) (OR=0,73; 95% CI: 0,63-0,84). Ein hohes Geburtsgewicht (> 4000 g), verglichen mit einem Geburtsgewicht < 4000 g, ist dagegen mit einem erhöhten Risiko für Übergewicht im späteren Leben assoziiert (OR=1,66; 95% CI: 1,55-1,77), ebenso wie ein hohes Geburtsgewicht (> 4000 g), verglichen mit einem normalen Geburtsgewicht (2500-4000 g) (OR=1,60; 95% CI: 1,45-1,77).

Der I^2 Test nach Higgins zeigte an, dass sowohl für zu niedriges Geburtsgewicht (I^2=93%) als auch für zu hohes Geburtsgewicht (I^2=81%) eine signifikante Heterogenität der Studienergebnisse vorlag. Die Sensitivitäts- und Subgruppenanalysen ergaben jedoch, dass die gepoolten Effektschätzer robust sind. Weder eine Stratifizierung nach Studiendesign oder nach der Methode der Geburtsgewichtserhebung gaben einen Hinweis auf 'recall bias'. Auch die geographische Herkunft der Studien, das Geschlecht der Studienteilnehmer oder die Publikationssprache hatten keinen Einfluss auf die Effektschätzer. Die Stratifizierung nach dem Alter ergab, dass es nach zu hohem Geburtsgewicht zu einer leichten Abschwächung des Übergewichtsrisikos im Laufe des Lebens kommt, jedoch im Erwachsenenalter noch immer eine 40%ige Risikoerhöhung nachweisbar ist (OR=1,40; 95% CI: 1,23-1,59), während nach geringem Geburtsgewicht keine signifikante Risikoverminderung mehr zu beobachten war. In der Stratifizierung nach dem Klassifikationskriterium für Übergewicht zeigte sich, dass Studien, die nicht Body-Mass-Index (BMI)-basierte Kriterien, wie bspw. den Taillenumfang oder den Taille-Hüft-Quotienten (engl. waist-to-hip-ratio, WHR), benutzt haben, sogar einen stärkeren Zusammenhang zwischen dem Geburtsgewicht und dem späteren Übergewichtsrisiko

beobachtet haben als Studien, die den BMI als Übergewichtskriterium benutzt hatten. Die 'influence analysis' ergab, dass die gepoolten Schätzer robust sind und keine der Einzelstudien den gepoolten Gesamtschätzer maßgeblich oder gar signifikant beeinflusst hatte.
Tests nach Begg und Egger lieferten weder für den Zusammenhang zwischen zu niedrigem Geburtsgewicht und späterem Übergewichtsrisiko (p=0,80 bzw. p=0,07) noch für den zwischen zu hohem Geburtsgewicht und Übergewichtsrisiko (p=0,45 bzw. p=0,23) im späteren Leben einen Hinweis auf das Vorliegen eines 'publication bias'. Der 'funnel plot' stellte sich symmetrisch dar und lieferte somit ebenfalls keinen Hinweis auf einen 'publication bias'.
Zur Beschreibung der Form des kontinuierlichen Zusammenhangs zwischen dem Geburtsgewicht und dem späteren Übergewichtsrisiko verwendeten wir die Methode der Metaregression. Nahezu über das gesamte Geburtsgewichtsspektrum hinweg zeigte sich ein linear positiver Zusammenhang zwischen dem Geburtsgewicht und dem späteren Übergewichtsrisiko. Bei einem Geburtsgewicht von unter 1500 g konnte keine weitere Risikoverminderung mehr beobachtet werden.
Für die Metaanalyse der confounder-adjustierten Daten wurden diejenigen Studien berücksichtigt, die adjustierte Schätzer für ein Geburtsgewicht < 2500 g und/oder > 4000 g berichtet haben. Während nur eine Studie einen adjustierten Schätzer für niedriges Geburtsgewicht angab, haben 16 der eingeschlossenen 66 Studien einen adjustierten Schätzer für hohes Geburtsgewicht berichtet. Der gepoolte adjustierte Schätzer für Übergewicht nach hohem Geburtsgewicht war ähnlich dem gepoolten Schätzer für die unadjustierten Daten derselben Studien mit den gleichen Referenzkategorien. Die Analyse der adjustierten Schätzer ergab, dass Kinder mit einem Geburtsgewicht von mehr als 4000 g in ihrem späteren Leben ein fast doppelt so hohes Risiko haben, übergewichtig zu werden wie normalgewichtige Neugeborene (OR=1,96; 95% CI: 1,43-2,67) unter Adjustierung auf Alter, Geschlecht, sozioökonomischen Status und elterliches Übergewicht.

1.4.2 Experimentelle Etablierung der Lasermikrodissektion

Im Experimentalansatz mit adulten Ratten führten 12 Stunden Nahrungskarenz zu einer deutlichen Abnahme der Leptin- und Insulinspiegel im Plasma (p<0,05), während die Blutglukosekonzentration nur tendenziell vermindert war. Hypoleptinämie und Hypoinsulinämie waren mit einer deutlich erhöhten Genexpression des orexigenen Neuropeptids AGRP im LMD-präparierten ARC assoziiert. Das Ausmaß der AGRP-Expression korrelierte dabei positiv mit der neuroanatomischen Spezifität des analysierten Untersuchungsmaterials von einer um 23% erhöhten Expression im Zellverband zu einer um 125% gesteigerten Expression in LMD-präparierten Einzelneuronen des ARC (jeweils p<0,05). Die Expression des anorexigenen Neuropeptids POMC war dagegen überraschenderweise unter Fastenbedingungen sowohl im Zellverband als auch in den Einzelneuronen des ARC unverändert (**Abb. 2**).

Die Applikation einer Glukoselösung führte bei Tieren, die zuvor einer Nahrungskarenz ausgesetzt waren, zu einem deutlichen Anstieg der Blutglukose (+137%) sowie zu einer deutlichen Hyperleptinämie (+608%) und Hyperinsulinämie (+544%) (alles $p<0,05$). Dementsprechend war die Expression des orexigenen AGRP im Zellverband herabreguliert und unterschied sich nun nicht mehr zwischen den Gruppen. In den präparierten Einzelneuronen war sogar eine signifikante Verminderung der AGRP-Genexpression (-50%) im Vergleich zu den Kontrolltieren zu beobachten. Dagegen war die Expression des anorexigenen Neuropeptids POMC nach Glukoseapplikation signifikant erhöht. Auch hier ließ sich mit zunehmender Spezifität des Untersuchungsmaterials eine deutliche Abstufung in der Expression beobachten (+44% im Zellverband, +128% in den Einzelneuronen; $p<0,05$, **Abb. 2**).

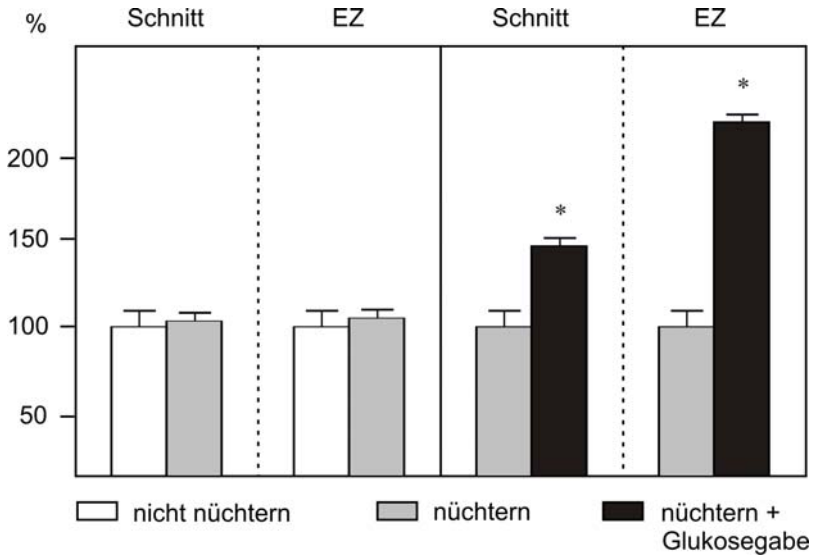

Abb. 2: mRNA-Expression von Proopiomelanocortin (POMC) im Nucleus arcuatus hypothalami (ARC) nach 12-stündiger Nahrungskarenz und nach peripherer Glukosegabe. Präparation des ARC als Zellverband (Schnitt) bzw. Einzelzellpool (EZ) [Landmann, *Schellong et al.* 2012]. Dargestellt ist die relative Genexpression bezogen auf beta Actin, angegeben als MW ± SEM in Prozent der Kontrollgruppenwerte. Gruppenvergleich mittels Mann-Whitney U-Test: * $p<0,05$.

1.4.3 Tierexperimentelle Langzeituntersuchungen zum 'small-baby-syndrome'

Körpergewicht

SGA-Tiere, die einer neonatalen Überernährung ausgesetzt waren (SGA-in-KW), zeigten frühpostnatal ein rasches Aufholwachstum und unterschieden sich bereits ab dem 7. LT nicht mehr signifikant von den Kontrolltieren (AGA-in-NW). Dagegen holten SGA-Tiere, die neonatal normal ernährt wurden (SGA-in-NW), dieses Gewichtsdefizit erst bis zum 60. LT auf. Nach dem 60. LT zeigten sich keine Unterschiede mehr zwischen den drei Gruppen, weder im Körpergewicht noch in der Körperlänge oder im relativen Körpergewicht. In der Analyse der Körperzusammensetzung bei Tötung am 560. LT ließ sich ein tendenziell erhöhter prozentualer Körperfettanteil nur bei den neonatal überernährten SGA-Tieren beobachten (n.s., **Abb. 3**).

Metabolische Parameter im adulten Alter

Während des gesamten Versuchszeitraumes konnten keine signifikanten Unterschiede der Nüchternblutglukose zwischen den Gruppen beobachtet werden. Im adulten Alter zeigten nur die neonatal überernährten SGA-Tiere (SGA-in-KW) sowohl unter normaler Ernährung (Standarddiät) am 360. LT als auch unter hyperkalorischer Hochfettdiät am 560. LT (bei Tötung) erhöhte Plasmaspiegel von Insulin ($p<0,05$) und Leptin ($p<0,05$ am 560. LT) sowie eine erhöhte Insulin/Blutglukose-Ratio ($p<0,05$) im Vergleich zu den Kontrolltieren (**Abb. 3**).

Nach intraperitonealer Glukosegabe im jungen adulten Alter (130. LT) zeigten neonatal überernährte SGA-Tiere (SGA-in-KW) eine signifikante Erhöhung der Blutglukose nach 90 Minuten ($p<0,05$), während die AUCG jedoch nicht signifikant erhöht war. Der GTT im hohen adulten Alter (530. LT) ergab keine signifikanten Unterschiede zwischen den Gruppen.

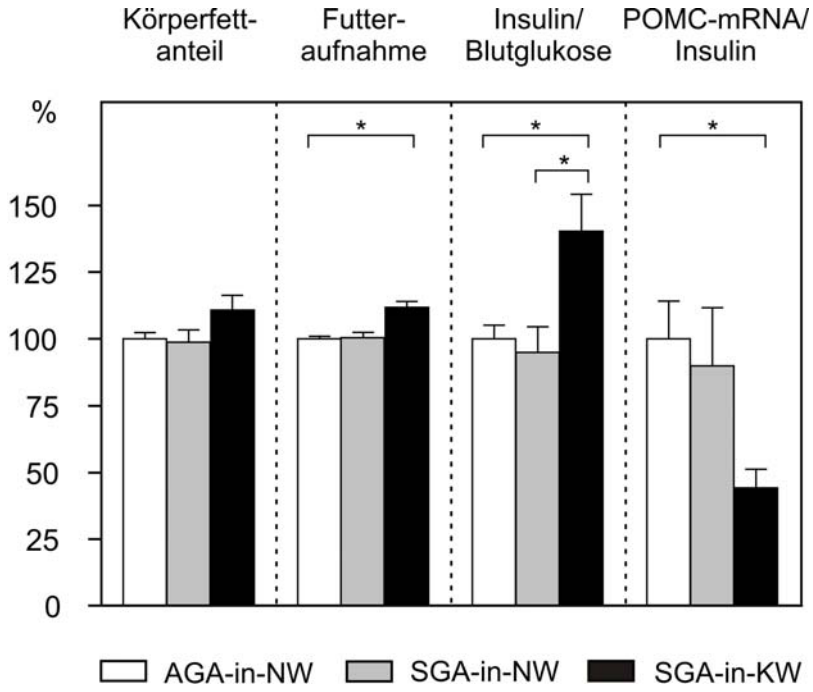

Abb. 3: Kernbefunde der tierexperimentellen Untersuchungen zum 'small-baby-syndrome' im adulten Alter. Dargestellt sind der Körperfettanteil am 560. LT, die Aufnahme von high-energy/high-fat-Diät im Zeitraum vom 500. bis 560. LT, die Insulin/Blutglukose-Ratio sowie der POMC-mRNA/Insulin-Quotient am 560. LT [*Schellong et al.* 2013]. Vergleich von Tieren mit normalem Geburtsgewicht in normalen Würfen (AGA-in-NW, weiße Säulen) mit Tieren, die ein vermindertes Geburtsgewicht aufwiesen und in normalen Würfen (SGA-in-NW, graue Säulen) bzw. kleinen Würfen (SGA-in-KW, schwarze Säulen) aufgezogen wurden. MW ± SEM, dargestellt in Prozent der Kontrollgruppenwerte. Gruppenvergleich mittels ANOVA und Tukeys HSD post-hoc-Analyse: * $p<0,05$.

Futteraufnahme im adulten Alter

In den Untersuchungen zur Futteraufnahme im adulten Alter zeigte sich vom 470. bis zum 500. LT eine signifikant verminderte Aufnahme von Standarddiät bei den neonatal normal ernährten SGA-Tieren (SGA-in-NW) sowohl im Vergleich zu den neonatal überernährten SGA-Tieren (SGA-in-KW; $p<0,05$) als auch im Vergleich zu den Kontrolltieren (AGA-in-NW; $p<0,01$).

SGA-in-NW-Tiere nahmen vom 500. bis 560. LT gleiche Mengen der hochkalorischen HE/HF-Diät auf wie AGA-in-NW-Tiere, während neonatal überernährte SGA-Tiere eine signifikant erhöhte Aufnahme von HE/HF-Diät zeigten ($p<0,01$, **Abb. 3**). Über den gesamten Beobachtungszeitraum vom 470. bis 560. LT zeigten die neonatal überernährten SGA-Tiere eine signifikant erhöhte Futter- und somit Energieaufnahme ($p<0,05$), wobei die mittlere tägliche Gesamtenergieaufnahme positiv mit dem prozentualen Körperfettgehalt der Tiere am 560. LT korrelierte ($r=0,24$; $p<0,05$).

Neuropeptidexpression im Nucleus arcuatus hypothalami (ARC)

In der Analyse der Neuropeptid-mRNA-Expression in Einzelneuronen des ARC am 560. LT konnten keine signifikanten Unterschiede zwischen den Gruppen festgestellt werden. Allerdings spiegelte sich bei den neonatal überernährten SGA-Tieren die signifikant erhöhte Nahrungsaufnahme vom 470. bis 560. LT in einer tendenziell verminderten POMC-Expression und einem Trend zu einer erhöhten Expression des orexigenen NPY im Vergleich zu den Kontrolltieren wider. SGA-Tiere, die neonatal normal ernährt wurden, zeigten dagegen sogar eine tendenziell verminderte Expression der orexigenen Neuropeptide NPY, AGRP und Galanin.
Da die hypothalamische Expression der Neuropeptide entscheidend von den im Blut zirkulierenden Sättigungssignalen abhängig ist [Schwartz *et al.* 1997, Woods *et al.* 2008], wurde ein Quotient aus der mRNA-Expression des jeweiligen Neuropeptids und den Plasmaspiegeln von Leptin bzw. Insulin gebildet [Plagemann *et al.* 2009]. Dabei zeigte sich eine signifikante Reduktion des POMC-mRNA/Insulin-Quotienten ($p<0,05$, **Abb. 3**) und eine tendenzielle Verminderung des POMC-mRNA/Leptin-Quotienten bei den neonatal überernährten SGA-Tieren (SGA-in-KW) gegenüber den Kontrolltieren. Bei den neonatal normal ernährten SGA-Tieren (SGA-in-NW) blieb auch unter Bezug auf die peripheren Sättigungssignale der Trend zu einer Down-Regulation der orexigenen Neuropeptide bestehen, was sich u.a. in einem signifikant verminderten AGRP-mRNA/Leptin-Quotienten zeigte ($p<0,05$).
Da die Regulation der Nahrungsaufnahme zudem ein Zusammenspiel orexigen und anorexigen wirkender Neuropeptide darstellt, führten wir hier erstmals die Berechnung eines Quotienten aus orexigener (NPY, AGRP, GAL) pro anorexigener (POMC) mRNA-Expression ein [*Schellong et al.* 2013]. Man erhält somit einen Netto-Indikator als integrativen Parameter der neuropeptidergen Sättigungsregulation. Neonatal überernährte SGA-Tiere wiesen dabei einen nahezu doppelt so hohen NPY/POMC-Quotienten auf sowohl im Vergleich zu SGA-in-NW-Tieren als auch im Vergleich zu den Kontrolltieren ($p<0,05$), der zudem positiv mit der Futteraufnahme korrelierte ($r=0,64$, $p<0,01$). Ebenfalls signifikant erhöht war der GAL/POMC-Quotient bei den neonatal überernährten SGA-Tieren im Vergleich zu den SGA-in-NW-Tieren ($p<0,05$).

1.5 Diskussion

Ausgehend von der zentralen Bedeutung von Übergewicht und Adipositas im Symptomenkomplex des metabolischen Syndroms [Cameron *et al.* 2008, Eckel *et al.* 2010] und unter Zugrundelegung der Hypothese des 'small-baby-syndrome' müsste das erhöhte Risiko für die Entwicklung eines metabolischen Syndroms bei untergewichtigen Neugeborenen maßgeblich über ein vermehrtes Auftreten von Übergewicht bzw. Adipositas im Kindes- bzw. Erwachsenenalter vermittelt werden. Dazu untersuchten wir im epidemiologischen Teil dieser Arbeit mit Hilfe einer Metaanalyse der bis *dato* publizierten Literatur, ob tatsächlich ein Zusammenhang zwischen dem Geburtsgewicht und dem Risiko für die Entwicklung von Übergewicht im späteren Leben besteht [*Schellong et al.* 2012]. Über nahezu das gesamte Geburtsgewichtsspektrum hinweg zeigte sich ein linear *positiver* Zusammenhang zwischen dem Geburtsgewicht und dem späteren Übergewichtsrisiko. Kinder, deren Geburtsgewicht über 4000 g lag, hatten ein nahezu verdoppeltes Übergewichtsrisiko im späteren Leben, unabhängig vom Geschlecht, dem Alter, dem sozioökonomischen Status und dem parentalen BMI. Dagegen stellte ein vermindertes Geburtsgewicht (< 2500 g) keinen Risikofaktor für Übergewicht im späteren Leben dar, wie in der Hypothese zum 'small-baby-syndrome' ursprünglich postuliert. Die vorliegende Metaanalyse ist mit 66 eingeschlossenen Studien, die mehr als 640.000 Studienteilnehmer aller Altersgruppen enthielten, die bisher umfassendste, globale Datensynthese zu diesem Thema. Andere Metaanalysen, die zeitgleich mit dieser bzw. unmittelbar danach entstanden sind, kamen ebenfalls zu dem Ergebnis, dass ein zu hohes Geburtsgewicht mit einem erhöhten Übergewichtsrisiko im späteren Leben assoziiert ist [Weng *et al.* 2012, Yu *et al.* 2011, Zhao *et al.* 2012].

An dieser Stelle sei kritisch angemerkt, dass in den meisten der in die Metaanalyse eingeschlossenen Studien der BMI als Klassifikationskriterium für Übergewicht/Adipositas verwendet worden ist. Obwohl sich die Ermittlung des BMI (kg/m^2) aufgrund seiner einfachen Handhabung als Routineparameter zur Übergewichtsklassifikation durchgesetzt hat, ist er für eine Risikoabschätzung übergewichtsassoziierter Morbiditäten, wie bspw. kardiovaskuläre Erkrankungen, aufgrund der Außerachtlassung des Muskel-Fett-Verhältnisses nur bedingt geeignet [Lee *et al.* 2008]. In zukünftigen Studien sollten daher Parameter gewählt werden, die eine Aussage über das Fettverteilungsmuster erlauben, wie etwa die WHR oder der Taillenumfang [Lee *et al.* 2008]. In einer Subgruppenanalyse von Studien, die nicht BMI-basierte Kriterien, wie bspw. die WHR oder den Taillenumfang, als Übergewichtskriterium benutzt haben, zeigte sich hier allerdings sogar ein stärkerer Zusammenhang zwischen dem Geburtsgewicht und dem späteren Übergewichtsrisiko als in Studien, die den BMI verwendet hatten, was einerseits die Rolle der WHR bzw. des Taillenumfangs als bessere Prädiktoren hervorhebt [Lee *et al.* 2008], andererseits die pathophysiologische Validität der hier gewonnenen Ergebnisse unterstreicht.

In den letzten Jahren wurde für den Effekt des verminderten Geburtsgewichtes auf spätere Krankheitsrisiken zunehmend die Rolle eines schnellen Aufholwachstums im Sinne eines 'rapid neonatal weight gain' anerkannt, wie von unserer Arbeitsgruppe seit langem postuliert [Dörner und Plagemann 1994]. So haben viele epidemiologische Studien gezeigt, dass eine rasche Gewichtszunahme bei Neugeborenen mit einem verminderten Geburtsgewicht einen Risikofaktor für die spätere Entwicklung von Adipositas, erhöhtem Körperfettanteil, gestörter Glukosetoleranz und kardiovaskulären Erkrankungen darstellt [Crowther *et al.* 1998, Fabricius-Bjerre *et al.* 2011, Forsén *et al.* 1999, Ibánez *et al.* 2006, Ong *et al*. 2000]. Dieser Zusammenhang ist sogar unabhängig vom Geburtsgewicht nachweisbar, d.h. über das gesamte Geburtsgewichtsspektrum [Stettler *et al.* 2002, Plagemann *et al.* 2012a].

In der vorliegenden Metaanalyse war eine stringente Subgruppenanalyse bezüglich der neonatalen Gewichtsentwicklung nicht durchführbar, da nur in 5 Originalstudien entsprechende Angaben gemacht worden sind, von denen wiederum nur 3 Studien adjustierte Schätzer beinhalteten. Keine dieser Studien berichtete allerdings ein erhöhtes Übergewichtsrisiko nach vermindertem Geburtsgewicht, weder in der Analyse der adjustierten noch in derjenigen der unadjustierten Daten. Im Gegenteil, jede dieser Studien zeigte eine positive Assoziation zwischen dem Geburtsgewicht und dem späteren Übergewichtsrisiko, auch unabhängig von der neonatalen Gewichtszunahme.

Als Ursache für eine gesteigerte frühkindliche Gewichtszunahme und deren Langzeitfolgen wird seit langem von unserer Arbeitsgruppe als auch zunehmend international einer neonatalen Überernährung maßgebliche Bedeutung beigemessen. Dass die frühpostnatale Überernährung eine entscheidende Rolle in der Entwicklung von Übergewicht, Insulinresistenz und weiteren Symptomen des metabolischen Syndroms darstellt, konnten unsere und weitere Arbeitsgruppen in zahlreichen Untersuchungen im Tiermodell zeigen. Werden bspw. normalgewichtige Ratten in kleinen Würfen ('Nestern') vom 3. bis 21. Lebenstag überernährt, so zeigen diese Tiere zunächst eine gesteigerte frühpostnatale Gewichtszunahme und entwickeln nachfolgend eine Reihe von Stoffwechselstörungen wie z.B. Hyperphagie, Insulinresistenz, Hyperleptinämie sowie einen erhöhten Körperfettgehalt [Boullu-Ciocca *et al.* 2005, Hou *et al.* 2011, López *et al.* 2005, Plagemann *et al.* 1999a und 1999b]. Die Ursachen für die beschriebene Symptomatik sind auf molekularbiologischer Ebene zu suchen. Frühere Arbeiten unserer Arbeitsgruppe zeigen, dass ein neonataler Hyperinsulinismus bzw. Hyperleptinismus infolge einer frühpostnatalen Überernährung zu einer neuroendokrinen Fehlprogrammierung von Regulationsmechanismen der Nahrungsaufnahme, des Körpergewichtes und des Stoffwechsels führen kann. Bei normalgewichtigen neonatal überernährten Ratten war die Genexpression der orexigenen, d.h. die Nahrungsaufnahme stimulierenden Neuropeptide NPY und GAL im ARC trotz hoher Leptin- und Insulinspiegel erhöht, während die Expression des anorexigenen, d.h. die Nahrungsaufnahme hemmenden POMC vermindert war, was zu einer lebenslang anhaltenden

Hyperphagie dieser Tiere beitragen kann [Plagemann *et al.* 1999a und 1999b und 2009, López *et al.* 2005].

Im tierexperimentellen Teil des Promotionsprojektes sollte deshalb mit Hilfe eines 'genuinen', d.h. kausal unabhängigen, hier neu etablierten Tiermodells für das 'small-baby-syndrome' im Langzeitversuch untersucht werden, inwieweit die neonatale Gewichtszunahme infolge neonataler Überernährung einen Einfluss auf das spätere Übergewichtsrisiko nach neonatalem Untergewicht hat. Hierzu wurden Ratten mit einem verminderten Geburtsgewicht vom 3. bis zum 21. LT durch Aufzucht in normalen Würfen ('Nestern') mit 12 Nachkommen pro Mutter normal ernährt oder in kleinen Würfen mit nur 3 Tieren aufgezogen, was zu einer qualitativen und quantitativen neonatalen Überernährung dieser Tiere führte [Babický *et al.* 1973, Fiorotto *et al.* 1991]. Hierbei zeigte sich, dass bei Tieren mit einem verminderten Geburtsgewicht eine Überernährung in den ersten Lebenstagen zu einer verstärkten neonatalen Gewichtszunahme führte im Gegensatz zu SGA-Tieren, die neonatal normal ernährt wurden. Obwohl sich die Tiere im adulten Alter hinsichtlich ihres Körpergewichtes nicht mehr unterschieden, zeigten die Tiere mit einem verminderten Geburtsgewicht im adulten Alter eine Disposition zu diabetogenen Stoffwechselstörungen, jedoch nur dann, wenn sie neonatal überernährt worden waren [*Schellong et al.* 2013].

In epidemiologischen Studien hat sich gezeigt, dass ca. 80-90% der SGA-Neugeborenen ihr Gewichtsdefizit innerhalb der ersten zwei Lebensjahre aufholen, während die restlichen 10-20% kein Aufholwachstum zeigen [Hokken-Koelega *et al.* 1995, Albertsson-Wikland und Karlberg 1994]. Eben diese rasche Gewichtszunahme stellt jedoch offenbar einen Risikofaktor für die spätere Entwicklung einer Insulinresistenz bei Kindern mit vermindertem Geburtsgewicht dar [Fabricius-Bjerre *et al.* 2011, Ibánez *et al.* 2006], die auch mit einer erhöhten abdominellen Fettmasse assoziiert ist [Ibánez *et al.* 2006].

Gemäß der 'Barker-Hypothese' haben Neugeborene mit einem verminderten Geburtsgewicht *per se* ein erhöhtes Risiko, im Laufe ihres Lebens Stoffwechselstörungen im Sinne eines metabolischen Syndroms, wie z.B. Diabetes mellitus Typ 2 oder Hyperlipidämie, zu entwickeln [Barker *et al.* 1993]. In der vorliegenden Studie zeigten die SGA-Tiere, die neonatal normal ernährt wurden, jedoch zu keinem Zeitpunkt metabolische Alterationen im Sinne eines erhöhten diabetogenen Risikos. Dagegen zeigten SGA-Tiere, die neonatal überernährt wurden (SGA-in-KW), im adulten Alter eine Hyperinsulinämie und eine erhöhte Insulin/Blutglukose-Ratio als Hinweise auf eine Insulinresistenz. Das spricht dafür, dass die beobachteten metabolischen Veränderungen bei SGA-in-KW-Tieren nicht auf das verminderte Geburtsgewicht zurückzuführen sind, sondern ihre Ursache eher in der frühpostnatalen Überernährung haben dürften.

Im Zusammenhang mit der Pathogenese des 'small-baby-syndrome' wurde in einer kürzlich erschienenen prospektiven Kohortenstudie eine dauerhaft veränderte Nahrungsaufnahme als

möglicher ’link’ zwischen einem verminderten Geburtsgewicht und dem späteren Auftreten von chronischen Krankheiten, wie z.B. Typ 2 Diabetes mellitus, diskutiert. In dieser Studie konnte gezeigt werden, dass bei Personen mit einem verminderten Geburtsgewicht im hohen adulten Alter die Aufnahme von Fett erhöht war [Perälä *et al.* 2012]. Dies bestätigt den Befund einer weiteren Longitudinal-Studie, in der bei Kindern eine inverse Beziehung zwischen dem Geburtsgewicht und der Fettaufnahme im Alter von 43 Monaten beschrieben wurde [Shultis *et al.* 2005]. Diese Beobachtungen konnten in der hier vorliegenden tierexperimentellen Studie bestätigt werden. Neonatal überernährte SGA-Tiere entwickelten eine Hyperphagie, insbesondere unter HE/HF-Diät. Diese könnte ursächlich für den tendenziell erhöhten Körperfettanteil dieser Tiere sein, was durch eine signifikant positive Korrelation zwischen Körperfett und Futteraufnahme bekräftigt wird. Allerdings zeigten neonatal normal ernährte SGA-in-NW-Tiere keine Hyperphagie, weder unter Standardfutter noch unter HE/HF-Diät. Insofern könnte für die epidemiologisch beobachtete ’Fettpräferenz’ nach low birth weight eher eine neonatale Überfütterung mit ’rapid neonatal weight gain’ verantwortlich sein, was in den genannten Studien allerdings nicht berücksichtigt und untersucht wurde.

Bemerkenswerterweise ergaben die LMD-basierten Genexpressionsanalysen in Einzelneuronen des ARC bei den neonatal überernährten SGA-in-KW-Tieren im adulten Alter, trotz ausgeprägter Hyperleptinämie und Hyperinsulinämie, im Vergleich zu den Kontrolltieren eine unveränderte Expression der orexigenen Neuropeptide NPY, GAL und AGRP. Dagegen war die Expression des anorexigenen POMC, auch unter Bezug auf die regulierenden peripheren Sättigungssignale Leptin und Insulin, vermindert. Insgesamt spricht dieses Expressionsmuster für eine neonatal erworbene Fehlprogrammierung des anorexigenen POMC-Systems infolge frühpostnataler Überernährung. Diese könnte der veränderten Nahrungsaufnahme der neonatal überernährten SGA-Tiere zugrunde liegen.

Die Bedeutung des anorexigenen POMC-Systems in der hypothalamischen Regulation von Nahrungsaufnahme und Stoffwechsel konnte zuvor im Zuge der experimentell basierten Etablierung der LMD-Methodik in dieser Promotionsarbeit bereits genauer charakterisiert werden. Hierzu untersuchten wir die Auswirkungen einer Nahrungskarenz auf die Genexpression orexigener und anorexigener Neuropeptide im ARC der Ratte. Unter Nahrungskarenz kam es, wie auch in der Literatur beschrieben [Sánchez *et al.* 2008], tatsächlich zu einer Up-Regulation des orexigenen Neuropeptids AGRP. Das Ausmaß der AGRP-Expression korrelierte dabei mit der neuroanatomischen Spezifität des Untersuchungsmaterials, also vom als Zellverband isolierten ARC bis hin zu den 100 gepoolten Einzelneuronen [Landmann, *Schellong et al.* 2012]. Überraschenderweise war die Expression des anorexigenen POMC, zu der in der Literatur widersprüchliche Befunde existierten [Ahima *et al*. 1999, Bi *et al.* 2003, Dallman *et al.* 1999, Palou *et al.* 2009, Pritchard *et al.* 2003], jedoch zugleich nicht down-reguliert. Sowohl im Zellverband als auch in den Einzelneuronen des ARC war die POMC-Expression unverändert,

trotz verminderter Leptin- und Insulinspiegel. Erst nach peripherer Glukoseapplikation kam es zu einer deutlichen Up-Regulation der POMC-Expression. Diese Beobachtungen weisen darauf hin, dass die POMC-Expression im ARC nicht nur oder in erster Linie leptin- bzw. insulinabhängig reguliert wird, sondern offenbar glukosevermittelt erfolgt. Im Sinne einer Gegenregulation, unter Glukose-mobilisierenden Situationen (wie z.B. Stress), scheint POMC im ARC eher als 'anti-orexigenes' Neuropeptid zu fungieren, ein Befund, der jenseits der hier realisierten Methodenetablierung einen maßgeblichen Beitrag zum Verständnis der Physiologie des 'POMC-Systems' insgesamt darstellt.

Darüber hinaus unterstützen diese Befunde zugleich die Genexpressionsdaten, die im Rahmen des 'genuinen' Tiermodells für das 'small-baby-syndrome' erhoben wurden. Hier zeigten neonatal überernährte SGA-Tiere im Vergleich zu den Kontrolltieren nahezu unveränderte Blutglukosespiegel. Vor dem Hintergrund des oben beschriebenen Einflusses der peripheren Glukose auf die hypothalamische POMC-Expression würde man hier folglich keine Regulation der POMC-Expression erwarten. Bemerkenswerterweise zeigten neonatal überernährte SGA-Tiere aber eine deutliche Down-Regulation des POMC im ARC, was für eine Fehlprogrammierung des anorexigenen POMC-Systems bei diesen Tieren spricht.

Mit den hier durchgeführten Untersuchungen ist es somit gelungen, die Methodenkombination aus Lasermikrodissektion und mRNA-Expressionsanalysen zu etablieren, die Methodenvalidität und Methodenspezifität zu verifizieren und zudem neue Erkenntnisse zur glukoseabhängigen POMC-Expression im Hypothalamus zu gewinnen.

1.6 Fazit

Die Ergebnisse dieser Promotionsarbeit zeigen, dass das alleinige Vorliegen eines verminderten Geburtsgewichtes keinen *unabhängigen* Risikofaktor für die Entwicklung eines Übergewichts und metabolischen Syndroms im späteren Leben darstellt, sondern dass vermutlich eine neonatale Überernährung nach niedrigem Geburtsgewicht eine entscheidende Rolle in der Pathogenese des 'small-baby-syndrome' spielen dürfte. Dabei legen die experimentellen Daten als möglichen Pathogenesemechanismus eine neuropeptiderge Fehlprogrammierung von Sättigungssignalen der Stoffwechsel- und Körpergewichtsregulation nahe. In Abbildung 4 sind die hier epidemiologisch und tierexperimentell gewonnenen Erkenntnisse zusammenfassend dargestellt und interpretiert (**Abb. 4**).

Das Geburtsgewicht wird nicht in erster Linie genetisch [Kilpeläinen *et al.* 2011], sondern maßgeblich durch das Intrauterinmilieu determiniert [Brooks *et al.* 1995], welches wiederum entscheidend durch die Ernährung bzw. den Ernährungszustand der Mutter beeinflusst wird. Mit der eingangs erwähnten, global zunehmenden Übergewichtsprävalenz steigt auch die Zahl übergewichtiger Frauen im reproduktionsfähigen Alter [Heslehurst *et al.* 2007]. Zahlreiche epidemiologische Studien zeigen mittlerweile, dass eine mütterliche prägravide Adipositas, eine

hohe Gewichtszunahme der Mutter in der Schwangerschaft sowie eine diabetische Stoffwechsellage *in graviditate* über eine fetale Überernährung zu einem erhöhten Makrosomierisiko des Kindes führen [Yu *et al.* 2013, Oken *et al.* 2009, Crowther *et al.* 2005] und nachfolgend zu einem erhöhten Übergewichtsrisiko im späteren Leben prädisponieren [*Schellong et al.* 2012]. Nach der Geburt wird die Gewichtszunahme des Neugeborenen vor allem durch die Säuglingsernährung bestimmt. Dabei stellt Stillen die optimale Form der Säuglingsernährung dar, weil es, neben einer Vielzahl weiterer Vorteile, auch das Risiko des Kindes für Übergewicht und damit assoziierte Stoffwechselstörungen im späteren Leben senkt und somit positive Langzeitwirkungen auf dessen Gesundheit hat [Gillman *et al.* 2001, Harder *et al.* 2005].

Die dargestellten Ergebnisse zeigen in übergreifender, translationaler Interpretation, dass sowohl pränatale als auch neonatale Einflüsse den sich entwickelnden Organismus prägen, dadurch einen lebenslangen Effekt auf dessen Gesundheit bzw. Krankheitsdispositionen haben können und somit geeignete Zeitfenster für primärpräventive Maßnahmen darstellen dürften, namentlich zur Vermeidung eines dauerhaft erhöhten Adipositas- und Diabetesrisikos.

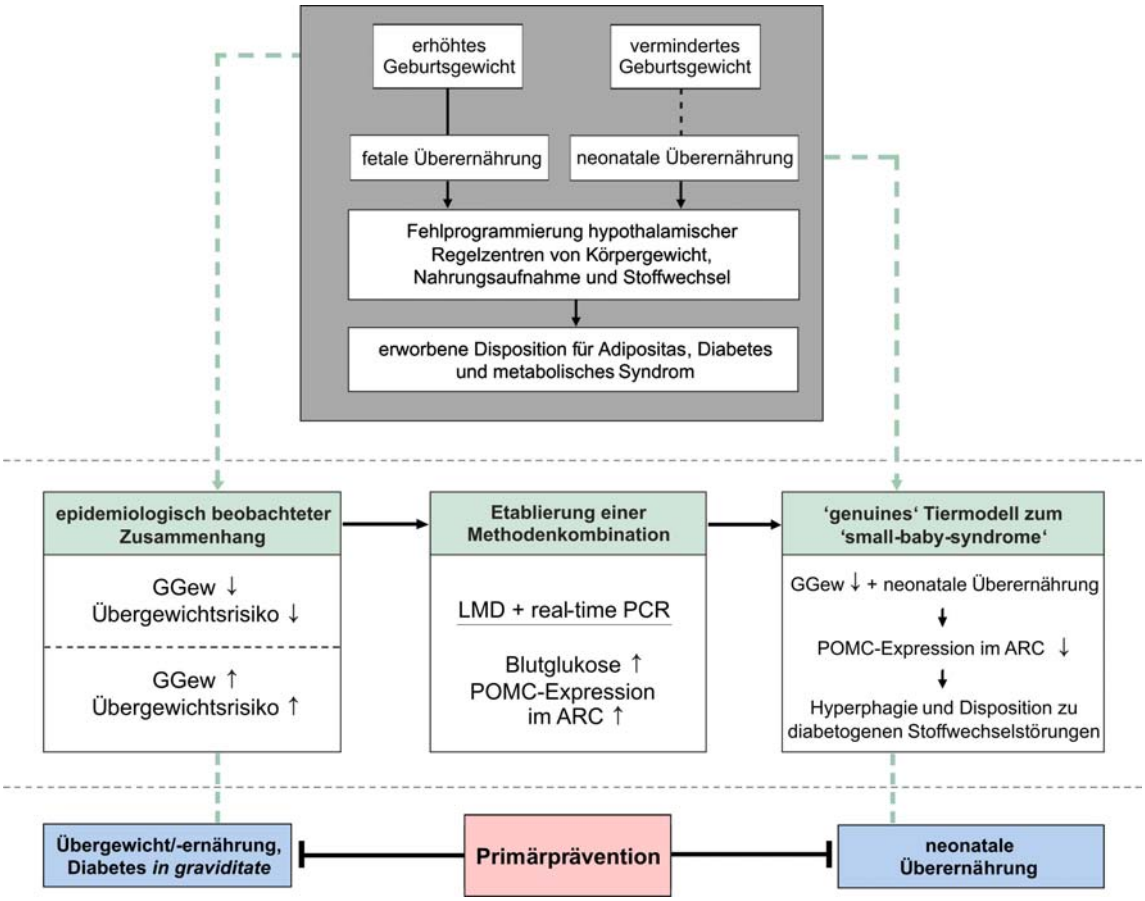

Abb. 4: Zusammenfassende Übersicht. Synopsis von Arbeitshypothese, translationalem Forschungsansatz und Kernbefunden der vorliegenden Publikationspromotion.
(ARC, Nucleus arcuatus hypothalami; GGew, Geburtsgewicht; LMD, Lasermikrodissektion; POMC, Proopiomelanocortin)

1.7 Literaturverzeichnis

Ahima RS, Kelly J, Elmquist JK, Flier JS. Distinct physiologic and neuronal responses to decreased leptin and mild hyperleptinaemia. Endocrinology 140 (1999) 4923-4931.

Albertsson-Wikland K, Karlberg J. Natural growth in children born small for gestational age with and without catch-up. Acta Paediatr 399 (1994) 64-70.

Babický A, Ostádalová I, Parízek J, Kolár J, Bíbr B. Onset and duration of the physiological weaning period for infant rats reared in nests of different sizes. Physiol Bohemoslov 22 (1973) 449-456.

Barker DJP, Hales CN, Fall CHD, Osmond C, Phipps K, Clarke PMS. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia 36 (1993) 62-67.

Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. Lancet 2 (1989) 577-580.

Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics 50 (1994) 1088-1101.

Berlin JA, Longnecker MP, Greenland S. Meta-analysis of epidemiologic dose-response data. Epidemiology 4 (1993) 218-228.

Bi S, Robinson BM, Moran TH. Acute food deprivation and chronic food restriction differentially affect hypothalamic NPY mRNA expression. Am J Physiol Regul Integr Comp Physiol 285 (2003) R1030-R1036.

Boullu-Ciocca S, Dutour A, Guillaume V, Achard V, Oliver C, Grino M. Postnatal diet-induced obesity in rats upregulates systemic and adipose tissue glucocorticoid metabolism during development and in adulthood. Diabetes 54 (2005) 197-203.

Brooks AA, Johnson MR, Steer PJ, Pawson ME, Abdalla HI. Birth weight: nature or nurture? Early Hum Dev 42 (1995) 29-35.

Cameron AJ, Boyko EJ, Sicree RA, Zimmet PZ, Söderberg S, Alberti KGMM, Tuomilehto J, Chitson P, Shaw JE. Central obesity as a precursor to the metabolic syndrome in the AusDiab study and Mauritius. Obesity 16 (2008) 2707-2716.

Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. N Engl J Med 352 (2005) 2477-2486.

Crowther NJ, Cameron N, Trusler J, Gray IP. Association between poor glucose tolerance and rapid post natal weight gain in seven-year-old children. Diabetologia 41 (1998) 1163-1167.

Dallman MF, Akana SF, Bhatnagar S, Bell ME, Choi S, Chu A, Horsley C, Levin N, Meijer O, Soriano LR, Strack AM, Viau V. Starvation: Early signals, sensors, and sequelae. Endocrinology 140 (1999) 4015-4023.

Dörner G, Plagemann A. Perinatal hyperinsulinism as possible predisposing factor for diabetes mellitus, obesity and enhanced cardiovascular risk in later life. Horm Metab Res 26 (1994) 213-221.

Eckel RH, Alberti KGMM, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 375 (2010) 181-183.

Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. Br Med J 315 (1997) 629-634.

Eriksson JG, Forsén T, Tuomilehto J, Winter PD, Osmond C, Barker DJP. Catch-up growth in childhood and death from coronary heart disease: longitudinal study. BMJ 318 (1999) 427-431.

Fabricius-Bjerre S, Jensen RB, Færch K, Larsen T, Mølgaard C, Michaelsen KF, Vaag A, Greisen G. Impact of birth weight and early infant weight gain on insulin resistance and associated cardiovascular risk factors in adolescence. PLoS One 6 (2011) e20595.

Fiorotto ML, Burrin DG, Perez M, Reeds PJ. Intake and use of milk nutrients by rat pups suckled in small, medium, or large litters. Am J Physiol 260 (1991) R1104-R1113.

Forsén T, Eriksson JG, Tuomilehto J, Osmond C, Barker DJP. Growth in utero and during childhood among women who develop coronary heart disease: longitudinal study. BMJ 319 (1999) 1403-1407.

Gamborg M, Byberg L, Rasmussen F, Andersen PK, Baker JL, Bengtsson C, Canoy D, Drøyvold W, Eriksson JG, Forsén T, Gunnarsdottir I, Järvelin MR, Koupil I, Lapidus L, Nilsen TI, Olsen SF, Schack-Nielsen L, Thorsdottir I, Tuomainen TP, Sørensen TI; NordNet Study Group. Birth weight and systolic blood pressure in adolescence and adulthood: Meta-regression analysis of sex- and age-specific results from 20 Nordic studies. Am J Epidemiol 166 (2007) 634-645.

Gillman MW, Rifas-Shiman SL, Camargo SA, Berkey CS, Frazier AL, Rocket HR, Field AE, Colditz GA. Risk of overweight among adolescents who were breastfed as infants. JAMA 285 (2001) 2461-2467.

Griffin M, Frazer A, Johnson A, Collins P, Owens D, Tomkin GH. Cellular cholesterol synthesis-The relationship to post-prandial glucose and insulin following weight loss. Atherosclerosis 138 (1998) 313-318.

Hales CN, Barker DJP. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia 35 (1992) 595-601.

Hales CN, Barker DJP. The thrifty phenotype hypothesis. Br Med Bull 60 (2001) 5-20.

Harder T, Bergmann R, Kallischnigg G, Plagemann A. Duration of breastfeeding and risk of overweight: a meta-analysis. Am J Epidemiol 162 (2005) 397-403.

Harder T, Kohlhoff R, Dörner G, Rohde W, Plagemann A. Perinatal 'programming' of insulin resistance in childhood: critical impact of neonatal insulin and low birth weight in a risk population. Diabetic Med 18 (2001) 634-639.

Harder T, Rodekamp E, Schellong K, Dudenhausen JW, Plagemann A. Birth weight and subsequent risk of type 2 diabetes: a meta-analysis. Am J Epidemiol 165 (2007a) 849-857.

Harder T, Schellong K, Stupin J, Dudenhausen JW, Plagemann A. Where is the evidence that low birth weight leads to subsequent obesity? Lancet 369 (2007b) 1859.

Haslam DW, James WPT. Obesity. Lancet 366 (2005) 1197-1209.

Heslehurst N, Ells LJ, Simpson H, Batterham A, Wilkinson J, Summerbell CD. Trends in maternal obesity incidence rates, demographic predictors, and health inequalities in 36,821 women over a 15-year period. BJOG 114 (2007) 187-194.

Higgins JP, Thompson SG, Deeks JJ, Altmann DG. Measuring inconsistency in meta-analysis. BMJ 327 (2003) 557-560.

Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL. Children born small for gestational age: do they catch up? Pediatr Res 38 (1995) 267-271.

Hossain P, Kawar B, El Nahas M. Obesity and diabetes in the developing world – a growing challenge. N Engl J Med 356 (2007) 213-215.

Hou M, Liu Y, Zhu L, Sun B, Guo M, Burén J, Li X. Neonatal overfeeding induced by small litter rearing causes altered glucocorticoid metabolism in rats. PLoS One 6 (2011) e25726.

Huxley R, Neil A, Collins R. Unravelling the fetal origins hypothesis: is there really an inverse association between birthweight and subsequent blood pressure? Lancet 360 (2002) 659-665.

Ibánez L, Ong K, Dunger DB, de Zegher F. Early development of adiposity and insulin resistance after catch-up weight gain in small-for-gestational-age children. J Clin Endocrinol Metab 91 (2006) 2153-2158.

Kelly T, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. Int J Obes 32 (2008) 1431-1437.

Kilpeläinen TO, den Hoed M, Ong KK, Grøntved A, Brage S, Early growth genetics consortium, Jameson K, Cooper C, Khaw KT, Ekelund U, Wareham NJ, Loos RJF. Obesity-susceptibility loci have a limited influence on birth weight: a meta-analysis of up to 28,219 individuals. Am J Clin Nutr 93 (2011) 851-860.

Knoll KP, Hauner H. Kosten der Adipositas in der Bundesrepublik Deutschland – Eine aktuelle Krankheitskostenstudie. Adipositas 2 (2008) 204-210.

Landmann EM, Schellong K, Melchior K, Rodekamp E, Ziska T, Harder T, Plagemann A. Short-term regulation of the hypothalamic melanocortinergic system under fasting and defined glucose-refeeding conditions in rats: A lasercapture microdissection (LMD)-based study. Neurosci Lett 515 (2012) 87-91.

Lee CMY, Huxley RR, Wildman RP, Woodward M. Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: a meta-analysis. J Clin Epidemiol 61 (2008) 646-653.

López M, Seoane LM, Tovar S, García MC, Nogueiras R, Diéguez C, Senarís RM. A possible role of neuropeptide Y, agouti-related protein and leptin receptor isoforms in hypothalamic programming by perinatal feeding in the rat. Diabetologia 48 (2005) 140-148.

Moher D, Liberati A, Tetzlaff J, Altmann DG, The PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA Statement. PLoS Med 6 (2009) e1000097.

Neitzke U, Harder T, Plagemann A. Intrauterine growth restriction and developmental programming of the metabolic syndrome: a critical appraisal. Microcirculation 18 (2011) 304-311.

Oken E, Kleinman KP, Belfort MB, Hammitt JK, Gillman MW. Associations of gestational weight gain with short- and longer-term maternal and child health outcomes. Am J Epidemiol 170 (2009) 173-180.

Ong KKL, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ 320 (2000) 967-971.

Palou M, Sánchez J, Rodriguez AM, Priego T, Picó C, Palou A. Induction of NPY/AgRP orexigenic peptide expression in rat hypothalamus is an early event in fasting: relationship with circulating leptin, insulin and glucose. Cell Physiol Biochem 23 (2009) 115-124.

Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates, 2. Aufl., Academic Press, San Diego, 1986.

Perälä MM, Männistö S, Kaartinen NE, Kajantie E, Osmond C, Barker DJP, Valsta LM, Eriksson JG. Body size at birth is associated with food and nutrient intake in adulthood. PLoS One 7 (2012) e46139.

Plagemann A. A matter of insulin: Developmental programming of body weight regulation. J Matern Fetal Neonatal Med 21 (2008) 143-148.

Plagemann A, Harder T, Brunn M, Harder A, Roepke K, Wittrock-Staar M, Ziska T, Schellong K, Rodekamp E, Melchior K, Dudenhausen JW. Hypothalamic proopiomelanocortin promoter methylation becomes altered by early overfeeding: an epigenetic model of obesity and the metabolic syndrome. J Physiol 587.20 (2009) 4963-4976.

Plagemann A, Harder T, Rake A, Voits M, Fink H, Rohde W, Dörner G. Perinatal elevation of hypothalamic insulin, acquired malformation of hypothalamic galaninergic neurons, and syndrome X-like alterations in adulthood of neonatally overfed rats. Brain Res 836 (1999a) 146-155.

Plagemann A, Harder T, Rake A, Waas T, Melchior K, Ziska T, Rohde W, Dörner G. Observations on the orexigenic hypothalamic neuropeptide Y-system in neonatally overfed weanling rats. J Neuroendocrinol 11 (1999b) 541-546.

Plagemann A, Harder T, Rodekamp E, Kohlhoff R. Rapid neonatal weight gain increases risk of childhood overweight in offspring of diabetic mothers. J Perinat Med 40 (2012a) 557-563.

Plagemann A, Harder T, Schellong K, Schulz S, Stupin JH. Early postnatal life as a critical time window for determination of long-term metabolic health. Best Pract Res Clin Endocrinol Metab 26 (2012b) 641-653.

Plagemann A, Roepke K, Harder T, Brunn M, Harder A, Wittrock-Staar M, Ziska T, Schellong K, Rodekamp E, Melchior K, Dudenhausen JW. Epigenetic malprogramming of the insulin receptor promoter due to developmental overfeeding. J Perinat Med 38 (2010) 393-400.

Pritchard LE, Oliver RL, McLoughlin JD, Birtles S, Lawrence CB, Turnbull AV, White A. Proopiomelanocortin-derived peptides in rat cerebrospinal fluid and hypothalamic extracts: evidence that secretion is regulated with respect to energy balance. Endocrinology 144 (2003) 760-766.

Sánchez E, Sánchez E, Singru PS, Acharya R, Bodria M, Fekete C, Zavacki A, Bianco AC, Lechan RM. Differential effects of refeeding on melanocortin-responsive neurons in the hypothalamic paraventricular nucleus. Endocrinology 149 (2008) 4329-4335.

Schellong K, Neumann U, Rancourt RC, Plagemann A. Increase of long-term ‘diabesity’ risk, hyperphagia, and altered hypothalamic neuropeptide expression in neonatally overnourished ‘small-for-gestational-age’ (SGA) rats. PLoS One 8 (2013) e78799.

Schellong K, Rodekamp E, Harder T, Dudenhausen JW, Plagemann A. Perinatale Prägung und lebenslange Krankheitsrisiken am Beispiel von Adipositas und Diabetes mellitus. Gynäkologe 41 (2008) 303-312.

Schellong K, Rodekamp E, Harder T, Dudenhausen JW, Plagemann A. Geburtsgewicht, Gestationsdiabetes und Perinatale Programmierung. CME Prakt Fortbild Gynäkol Geburtsmed Gynäkol Endokrinol 3 (2009) 182-192.

Schellong K, Schulz S, Harder T, Plagemann A. Birth weight and long-term overweight risk: systematic review and a meta-analysis including 643,902 persons from 66 studies and 26 countries globally. PLoS One 7 (2012) e47776.

Schmittgen T, Livak K. Analyzing real-time PCR data by the comparative Ct method. Nat Protocol 3 (2008) 1101-1108.

Schwartz MW, Seeley RJ, Woods SC, Weigle DS, Campfield LA, Burn P, Baskin DG. Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. Diabetes 46 (1997) 2119-2123.

Scully T. Diabetes in numbers. Nature 485 (2012) S2-S3.

Shultis WA, Leary SD, Ness AR, Bain CJ, Emmet PM, and the ALSPAC Study Team. Does birth weight predict childhood diet in the Avon longitudinal study of parents and children? J Epidemiol Community Health 59 (2005) 955-960.

Singhal A, Cole TJ, Fewtrell M, Kennedy K, Stephenson T, Elias-Jones A, Lucas A. Promotion of faster weight gain in infants born small for gestational age: is there an adverse effect on later blood pressure? Circulation 115 (2007) 213-220.

Stettler N, Zemel BS, Kumanyika S, Stallings VA. Infant weight gain and childhood overweight status in a multicenter, cohort study. Pediatrics 109 (2002) 194-199.

Weng SF, Redsell SA, Swift JA, Yang M, Glazebrook CP. Systematic review and meta-analyses of risk factors for childhood overweight identifiable during infancy. Arch Dis Child 97 (2012) 1019-1026.

Woods SC, D' Alessio DA. Central control of body weight and appetite. J Clin Endocrinol Metab 93 (2008) S37-S50.

Yu Z, Han S, Zhu J, Sun X, Ji C, Guo X. Pre-pregnancy body mass index in relation to infant birth weight and offspring overweight/obesity: A systematic review and meta-analysis. PLoS One 8 (2013) e61627.

Yu ZB, Han SP, Zhu GZ, Zhu C, Wang XJ, Cao XG, Guo XR. Birth weight and subsequent risk of obesity: a systematic review and meta-analysis. Obes Rev 12 (2011) 525-542.

Zhao Y, Wang SF, Mu M, Sheng J. Birth weight and overweight/obesity in adults: a meta-analysis. Eur J Pediatr 171 (2012) 1737-1746.

2. Eidesstattliche Versicherung

„Ich, Karen Schellong, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema:

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Datum Karen Schellong

3. Anteilserklärung

Frau Karen Schellong hatte folgenden Anteil an den vorgelegten Publikationen:

Publikation 1:

Schellong K*, Schulz S*, Harder T, Plagemann A. Birth weight and long-term overweight risk: systematic review and a meta-analysis including 643,902 persons from 66 studies and 26 countries globally. PLoS One 7 (2012) e47776. **IF: 3,730**

* geteilte Erstautorenschaft

Beitrag im Einzelnen:

Erstellung des Protokolls der Metaanalyse, Datenbankrecherche, Datenextraktion, Datenanalyse und Statistik, maßgebliche Beteiligung an Abfassung, Einreichung und Reviewprozess der Publikation

Publikation 2:

Landmann EM*, **Schellong K***, Melchior K, Rodekamp E, Ziska T, Harder T, Plagemann A. Short-term regulation of the hypothalamic melanocortinergic system under fasting and defined glucose-refeeding conditions in rats: A lasercapture microdissection (LMD)-based study. Neuroscience Letters 515 (2012) 87-91. **IF: 2,026**

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Beitrag im Einzelnen:

Histologische Präparationen (Lasermikrodissektion), molekularbiologische Arbeiten (RNA-Isolierung, cDNA-Synthese, quantitative real-time PCR), Datenauswertung und Statistik, Erstellen von Abbildungen, maßgebliche Beteiligung an Abfassung, Einreichung und Reviewprozess der Publikation

Publikation 3:

Schellong K, Neumann U, Rancourt RC, Plagemann A. Increase of long-term 'diabesity' risk, hyperphagia, and altered hypothalamic neuropeptide expression in neonatally overnourished 'small-for-gestational-age' (SGA) rats. PLoS One 8 (2013) e78799. **IF: 3,730**

Beitrag im Einzelnen:

Tierexperimentelle Untersuchungen (Körpergewichts- und Körperlängenmessung, Futterrückwagen), histologische Präparationen (Lasermikrodissektion), molekularbiologische Arbeiten (RNA-Isolierung, cDNA-Synthese, quantitative real-time PCR), Datenauswertung und Statistik, Erstellen von Abbildungen, maßgebliche Beteiligung an Abfassung, Einreichung und Reviewprozess der Publikation

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Datum Karen Schellong

4. Druckexemplare der ausgewählten Publikationen

- **Schellong K**, Schulz S, Harder T, Plagemann A. Birth weight and long-term overweight risk: systematic review and a meta-analysis including 643,902 persons from 66 studies and 26 countries globally. PLoS One 7 (2012) e47776.

- Landmann EM, **Schellong K**, Melchior K, Rodekamp E, Ziska T, Harder T, Plagemann A. Short-term regulation of the hypothalamic melanocortinergic system under fasting and defined glucose-refeeding conditions in rats: A lasercapture microdissection (LMD)-based study. Neuroscience Letters 515 (2012) 87-91.

- **Schellong K**, Neumann U, Rancourt RC, Plagemann A. Increase of long-term 'diabesity' risk, hyperphagia, and altered hypothalamic neuropeptide expression in neonatally overnourished 'small-for-gestational-age' (SGA) rats. PLoS One 8 (2013) e78799.

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Birth Weight and Long-Term Overweight Risk: Systematic Review and a Meta-Analysis Including 643,902 Persons from 66 Studies and 26 Countries Globally

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Abstract

Background: Overweight is among the major challenging health risk factors. It has been claimed that birth weight, being a critical indicator of prenatal developmental conditions, is related to long-term overweight risk. In order to check this important assumption of developmental and preventive medicine, we performed a systematic review and comprehensive meta-analysis.

Methods and Findings: Relevant studies published up to January 2011 that investigated the relation between birth weight and later risk of overweight were identified through literature searches using MEDLINE and EMBASE. For meta-analysis, 66 studies from 26 countries and five continents were identified to be eligible, including 643,902 persons aged 1 to 75 years. We constructed random-effects and fixed-effects models, performed subgroup-analyses, influence-analyses, assessed heterogeneity and publication bias, performed meta-regression analysis as well as analysis of confounder adjusted data. Meta-regression revealed a linear positive relationship between birth weight and later overweight risk ($p<0.001$). Low birth weight (<2,500 g) was found to be followed by a decreased risk of overweight (odds ratio (OR) = 0.67; 95% confidence interval (CI) 0.59–0.76). High birth weight (>4,000 g) was associated with increased risk of overweight (OR = 1.66; 95% CI 1.55–1.77). Results did not change significantly by using normal birth weight (2,500–4,000 g) as reference category (OR = 0.73, 95% CI 0.63–0.84, and OR = 1.60, 95% CI 1.45–1.77, respectively). Subgroup- and influence-analyses revealed no indication for bias/confounding. Adjusted estimates indicate a doubling of long-term overweight risk in high as compared to normal birth weight subjects (OR = 1.96, 95% CI 1.43–2.67).

Conclusions: Findings demonstrate that low birth weight is followed by a decreased long-term risk of overweight, while high birth weight predisposes for later overweight. Preventing *in-utero* overnutrition, *e.g.*, by avoiding maternal overnutrition, overweight and/or diabetes during pregnancy, might therefore be a promising strategy of genuine overweight prevention, globally.

Citation: Schellong K, Schulz S, Harder T, Plagemann A (2012) Birth Weight and Long-Term Overweight Risk: Systematic Review and a Meta-Analysis Including 643,902 Persons from 66 Studies and 26 Countries Globally. PLoS ONE 7(10): e47776. doi:10.1371/journal.pone.0047776

Editor: Adrian V. Hernandez, Universidad Peruana de Ciencias Aplicadas (UPC), Peru

Received July 19, 2012; **Accepted** September 17, 2012; **Published** October 17, 2012

Funding: This study was supported by grants of the the German Research Foundation (DFG: GRK 1208; PL 241/5-1) to Dr. Plagemann. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Overweight is among the top challenging health problems at the beginning of the 21st century [1]. Prevalence has increased alarmingly, reaching epidemic levels in adults, adolescents, and even children in the US and globally [1–3]. Across the age spectrum, critical metabolic and cardiovascular morbidity (type 2 diabetes, hypertension, metabolic syndrome, coronary heart disease, stroke) is causally linked to obesity [2,4,5]. Cardiovascular and all-cause mortality are strongly related to overweight and obesity irrespective of age, sex, and ethnicity [6–9]. Therefore, measures of primary, genuine prevention are urgently needed.

For some years, the 'fetal origins hypothesis' on early causes of later diseases has become one of the most promising theoretical frameworks in medicine [10–12]. Especially, birth weight has been suggested and used as basic indicator to establish these highly influential concepts [10,12], since it is decisively determined by the prenatal developmental conditions [13,14]. However, one of the most important axioms in this context has not been tested globally so far. Although overweight is of central pathogenetic importance for metabolic, cardiovascular and general morbidity and mortality [2,4,5], no comprehensive analysis has investigated whether the risk of becoming overweight is related to birth weight in children, adolescents, and adults, *i.e.*, for the long-term.

Therefore, we aimed to characterize this overall critical aspect of the 'fetal origins' approach by a systematic review and meta-

analysis, substantially extending previous preliminary reviews [15–17].

Methods

Search strategy and selection criteria

Systematic review and meta-analysis were conducted according to the PRISMA statement for meta-analysis of observational studies (Text S1) [18], including the preparation of a protocol and analysis plan (Text S2). We performed a comprehensive literature search, including the databases MEDLINE and EMBASE (1966–January 2011), to identify studies that investigated the relation between birth weight and later risk of overweight. Searched terms were "birth weight", "overweight", "obesity" and "adiposity", without language restrictions. Furthermore, we manually searched all references cited in original studies and all reviews identified. Authors were contacted if data, methods and/or parameter definitions provided from the respective studies remained unclear.

To be eligible for meta-analysis, a study had to fulfill the following criteria, defined *a priori*: 1) It had to be an original report on the relation between birth weight and risk of overweight. 2) Odds ratios (OR) and 95% confidence intervals (95% CI) (or data with which to calculate them) for risk of overweight in at least two strata of birth weight had to have been reported. All studies which reported the proportion of overweight or obese subjects in at least one age at follow up were included. We did not restrict to a particular definition of overweight/obesity as studies may have been published before currently accepted definitions were introduced [16]. The majority of studies used body mass index (BMI) as overweight criterion in childhood, adolescence as well as adulthood (80%).

From all eligible studies, data were abstracted in duplicate, using a standardized form. An independent reviewer confirmed all data entries.

Statistical analysis

Dichotomous comparisons. We extracted data on numbers of subjects with and without overweight above or below the cutoff value and calculated corresponding crude odds ratios and 95% confidence intervals. We constructed fixed-effects as well as random-effects models to estimate the pooled odds ratios for risk of overweight above *vs.* below the respective cutoff value across all studies.

Assessment of heterogeneity. By calculating the I^2 according to Higgins et al [19], we assessed heterogeneity. Ranging from 0 to 100%, I^2 is a direct measure of inconsistency of study results in a meta-analysis, with 0% indicating no inconsistency.

Influence analysis. Robustness of the pooled estimates was checked by influence analyses. Each of the studies was individually omitted from the data set, followed in each case by recalculation of the pooled estimate of the remaining studies.

Subgroup/Sensitivity analyses. To identify potential sources of heterogeneity and sources of bias, studies were stratified by study design and source of birth weight data to assess potential recall bias. Additionally, studies were stratified by publication language. To examine participation/selection bias, we stratified by extent of lost to follow-up. Further stratifications were made by geographic origin, age, overweight classification criterion, source of overweight data, gender distribution, gestational age and parental overweight (BMI>25 kg/m^2). To assess the impact of parental socioeconomic status (SES), we stratified by the extent by which low SES was present in the study samples.

Publication bias. Publication bias was assessed by inspection of the funnel plot and formal testing for funnel plot asymmetry, using Begg's test and Egger's test.

Meta-regression. To explore the shape of the continuous relation between birth weight and later overweight risk, meta-regression technique was applied [20]. Accordingly, birth weight-specific odds ratios were related to the respective birth weight. Since birth weight was reported as categorical data with a certain range in the studies (per example, 2,000–2,500 g, 3,000–3,500 g etc.), median of the upper and lower limits of each category was assigned to the particular estimate in each study [21]. Estimates were plotted against respective birth weight as independent variable. After visual inspection, we primarily decided to use a linear regression model. Additionally, fractional polynomial regression was applied because it does not make an *a priori* assumption on shape of the curve. The family of second-order fractional polynomial models provides rich and flexible shapes of curves by choosing $p = (p_1,\ldots,p_m)$ as real-valued vector of fractional power from a predefined set [22]. All estimates were weighted by 1/variance.

Analysis of confounder-adjusted data. To perform meta-analysis of confounder-adjusted data we considered all studies which reported adjusted odds ratios for risk of overweight for the birth weight categories <2,500 g and/or >4,000 g. Resulting pooled odds ratios were based, however, on different reference categories, as defined by the authors themselves, and therefore not directly comparable to the pooled unadjusted odds ratios evaluated for <2,500 g *vs.* >2,500 g and >4,000 g *vs.* <4,000 g, respectively. To make pooled adjusted and unadjusted odds ratios more comparable, we therefore additionally calculated in all studies which provided adjusted data an unadjusted odds ratio that considered the reference category as used self-chosen by the authors for the adjusted odds ratios in the respective studies (bottom Tab. 1). Thereby, an orientating comparability of pooled adjusted *vs.* unadjusted odds ratios over all eligible studies was achieved [23–25].

Software. All calculations were performed with STATA, version 11.0, software (Stata Corp., College Station, TX, USA).

Ethics Statement

An ethics statement was not required for this work.

Results

Baseline characteristics of the studies

Course of the systematic review is illustrated in a flow diagram according to the PRISMA statement (Figure 1). From a total of 3,513 potentially relevant entries, 108 studies were identified which related birth weight to risk of later overweight [26–133]. They involved a total of 1,485,561 persons at six months to 79 years of age (for study characteristics see Table S1).

In the systematic review, 94 of the 108 studies (87%) reported a positive relation between birth weight and later risk of overweight. In 7 studies (6.5%), no relation between birth weight and later risk of overweight was observed, while in 6 studies (5.6%) a U-shaped relation was found. Only one study (0.9%) reported an inverse relation between birth weight and later overweight risk.

For methodological reasons, 42 studies could not be included in the meta-analysis. Reasons for exclusion were firstly, data were insufficiently reported for quantitative analysis (n = 20). Secondly, birth weight was reported as z-score or centiles without units (n = 9) and thirdly, birth weight was dichotomized at unjustified cut points (n = 13) (see Figure 1). However, these excluded studies showed no relevant differences in general characteristics, *e.g.*,

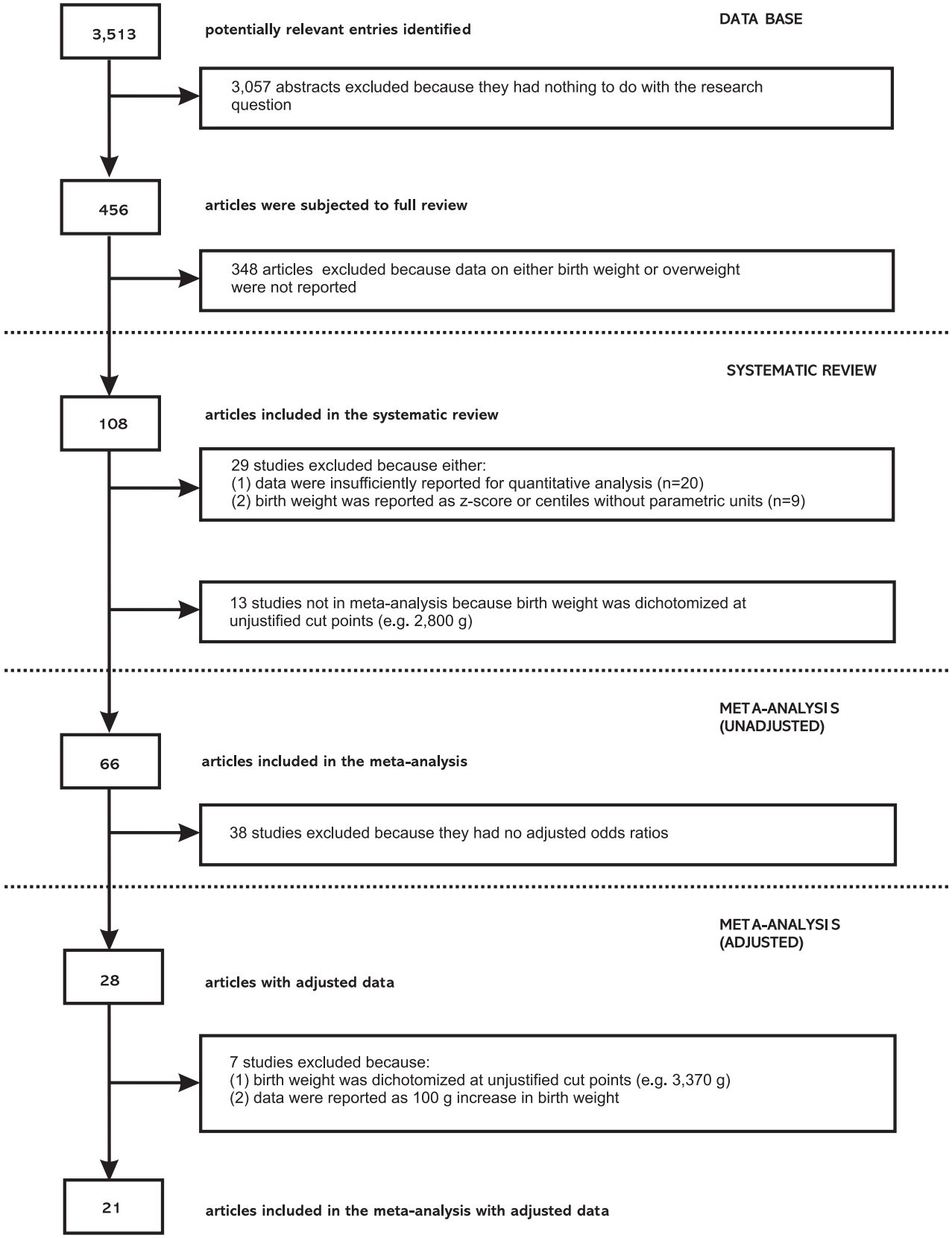

Figure 1. Flow diagram of selection process. Course of systematic literature review on birth weight and risk of overweight later in life, 1966–January 2011.
doi:10.1371/journal.pone.0047776.g001

distribution of geographic origin, age at follow up, assessment of overweight etc. Moreover, the observed relations between birth weight and later outcome did generally not differ from those observed in studies which could be included in the meta-analysis. Of the 42 excluded studies, 35 (83.4%) reported a positive relation between birth weight and later risk of overweight. In 3 studies (7.1%), no relation between birth weight and risk of later overweight was observed. A U-shaped relation was reported in 3 studies (7.1%) and only one study (2.4%) reported an inverse relation between birth weight and later overweight risk. These percentages were very similar to those observed in the studies which could be included into meta-analysis (see below).

For meta-analysis, 66 studies were identified to be eligible, including 58 cohort studies and eight studies with case-control-design [68–133], involving a total of 643,902 persons. Age of participants ranged from 1 to 75 years and the year of birth ranged from 1914–2004 (Table S1). Studies were performed in Asia, Australia, Europe, North America and South America. Study size varied ranging from 82 to 153,536 participants.

General estimates

Of the 66 studies eligible for meta-analysis 59 studies (89.4%) reported a positive relation between birth weight and later risk of overweight. In 4 studies (6.1%), no relation between birth weight and later risk of overweight was observed, whereas in 3 studies (4.5%) a U-shaped relation was detected. None of the studies reported an inverse relation between birth weight and later overweight risk.

Low birth weight (<2,500 g) was found to be associated with a decreased risk of overweight in the random-effects model (OR = 0.67; 95% CI: 0.59–0.76) as well as in the fixed-effects model (OR = 0.75; 95% CI: 0.72–0.79; see Figure 2).

High birth weight (>4,000 g) was associated with increased risk of overweight in the random-effects model (OR = 1.66; 95% CI: 1.55–1.77) as well as in the fixed-effects model (OR = 1.61; 95% CI: 1.57–1.65; see Figure 3).

Given these results, we repeated the dichotomous comparisons, now using "normal birth weight" (2,500–4,000 g) as reference category for all studies that gave data on both ends of the birth weight spectrum. The pooled estimate for low birth weight was 0.73 (95% CI: 0.63–0.84) and those for high birth weight was 1.60 (95% CI: 1.45–1.77; all random-effects model).

Influence analysis showed that the pooled estimates were robust. Omission of individual studies revealed that no single study had a particular influence on the pooled estimates, detected by pooled odds ratios ranging from 0.65 (95% CI: 0.57–0.73) to 0.68 (95% CI: 0.60–0.77) for low birth weight, and 1.64 (95% CI: 1.54–1.75) to 1.68 (95% CI: 1.58–1.78) for high birth weight.

Sensitivity/subgroup analyses

According to I^2 [19], results were heterogeneous for both low birth weight ($I^2 = 93\%$) and high birth weight ($I^2 = 81\%$). To identify possible sources of heterogeneity and bias, we performed sensitivity/subgroup analyses (Table 1). Neither stratification by study design, nor by method of recording birth weight gave indication of recall bias. Geographic origin had no impact on the pooled estimates. Stratification by publication language also gave no indication for respective bias. Consideration of age revealed that the effect of high birth weight remained significant even in adulthood, whereas influence of low birth weight remained non-significant. Stratification by overweight classification criterion showed that studies which used non-BMI-based criteria, such as waist circumference (WC), waist-to-hip ratio (WHR) or waist-to-height ratio (WHtR), reported even stronger relations between birth weight and later overweight risk. Gender distribution had no impact on the pooled estimates. Furthermore, studies with higher lost to follow-up rates (>20%) had similar pooled estimates as those with low percentage of lost participants (≤20%). Surprisingly, a considerable number of studies did not account for gestational age, SES and/or parental body weight. However, even these potentially critical confounders had no significant impact on the overall results (Table 1).

Impact of publication bias

Neither for the relation between low birth weight and risk of overweight nor for that between high birth weight and later overweight risk evidence for publication bias was found, as indicated by visual inspection of funnel plots, proven by nonsignificant Begg's tests (low birth weight: $p = 0.80$; high birth weight: $p = 0.45$) and Egger's tests (low birth weight: $p = 0.07$; high birth weight: $p = 0.23$).

Shape of the continuous association between birth weight and risk of overweight

In a first step, we fitted a linear meta-regression model to the data, which revealed a significant positive linear relation between birth weight and subsequent overweight risk ($b = 0.34 \times 10^{-3}$ ($0.28 - 0.40 \times 10 - 3$); $p < 0.001$). To further explore the shape of the association, fractional polynomial regression was used. Figure 4 shows the shape of the curve for the continuous relation between birth weight and later risk of overweight, estimated by a second-order fractional polynomial regression model ($b_1 = 14.13$ (9.27–19.00); $b_2 = -20.34$ ($-26.58 - (-14.09)$); $p_1 = -1$; $p_2 = -0.5$; $p < 0.001$; inverse variance weighted). Over nearly the entire birth weight spectrum, birth weight was found to be linearly positively related to overweight risk. Risk does not further decrease below a birth weight of 1,500 g. Comparison of model fitness parameters showed that a linear regression model performed as good as the fractional polynomial model ($p = 0.17$ for comparison of deviance between both models).

Impact of confounders on strength of the association

Whereas only one study gave an adjusted estimate for low birth weight, in 16 studies confounder-adjusted estimates for risk of overweight after high birth weight were reported. Number and types of adjustments varied across the studies (Table S2). The pooled confounder-adjusted estimate for overweight after high birth weight was nearly the same as the pooled estimate of the unadjusted data from the same studies using the same reference category (Table 1). Fully adjusted estimates revealed a nearly doubled long-term overweight risk in individuals with high birth weight compared to normal birth weight subjects (OR = 1.96; 95% CI: 1.43–2.67). Weighted fractional polynomial regression of the confounder-adjusted data revealed very similar results as in the case of unadjusted data ($b_1 = 16.59$ (9.55–23.64); $b_2 = -24.11$ (-33.06 (-15.16)); $p_1 = -1$; $p_2 = -0.5$; $p < 0.001$; inverse variance weighted).

Discussion

Over several years, increasing attention is given on early developmental origins of long-term diabetic, adipogenic, and cardiovascular disorders in terms of the metabolic syndrome [10,11]. However, the direction and strength of relation between birth weight and long-term overweight risk has not been tested globally so far in a comprehensive manner. Therefore, we aimed to characterize this overall critical aspect of the 'fetal origins' approach by a respective meta-analysis, quantitatively as well as

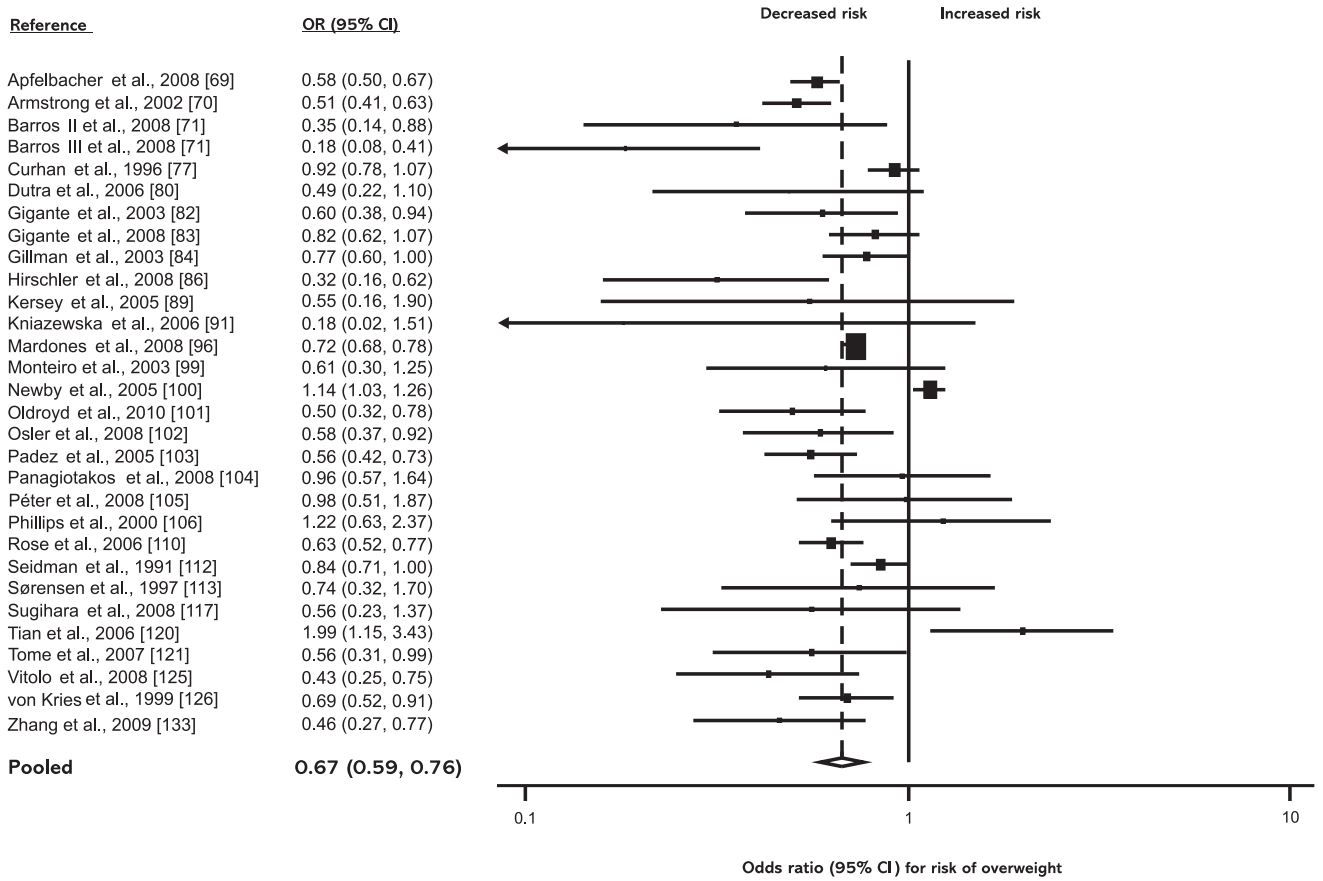

Figure 2. Low birth weight (<2,500 g) and subsequent risk of overweight. ORs for overweight in subjects with birth weights <2,500 g as compared with subjects with birth weights ≥2,500 g. Studies are ordered alphabetically by first author. The point estimate *(center of each black square)* and the statistical size *(proportional area of square)* are represented. Horizontal lines indicate 95% confidence intervals. The pooled odds ratio *(diamond)* was calculated by means of a random effects model. OR, odds ratio; CI, confidence interval.
doi:10.1371/journal.pone.0047776.g002

qualitatively substantially extending and proving previous preliminary reviews, statements, proposals etc. by our own group and others [15–17].

The data set and meta-analysis provided here is by far the largest one analyzed to date on this topic and the first to address the whole lifespan, provide confounder-adjusted estimates and incorporate global data *i.e.*, including western, westernized as well as developing countries in Northern- and South America, Europe, Asia and Australia. Our analysis shows with high consistency that an increased birth weight (>4,000 g) may lead to a doubling of the long-term overweight risk, irrespective of geographic/ethnic origin, sex, socio-economic status, parental weight status etc.

In the context of the 'small baby syndrome' and the respective 'fetal origins' hypotheses [10–12], it has been claimed that low birth weight is a risk factor for cardiovascular diseases and type 2 diabetes, as tested by systematic reviews and meta-analyses with mixed results [134–138], as well as for the development of overweight/obesity, *i.e.*, one of the most critical cardiometabolic risk determinants [5–7]. Interestingly, results of our meta-analysis do not support this claim. In contrast, across the birth weight spectrum a linear positive relation exists with later overweight risk. Only a small fraction of studies, analyzing probands with very low birth weight (VLBW; <1,500 g; n = 3 studies), found no further decrease of overweight risk at this very 'left-handed' side of the birth weight spectrum.

General concerns on our data and their interpretation might arise regarding the suitability of investigated parameters and/or the consideration of bias and/or confounding variables. For instance, it has increasingly been proposed that the effect of low birth weight on later health risks might rather result from increased ('rapid') neonatal weight gain/catch up growth [139]. This was regarded, unfortunately, in only five reports (4.6%; Table S3) [79,109,114,115,116], three of which [79,115,116] reported respectively adjusted data analyses. An 'obesogenic' effect of low birth weight was not observed in these unadjusted or adjusted studies. By contrast, all found an effect of high birth weight on later overweight risk, independently of early weight gain. Furthermore, although BMI has been established as routine parameter to identify overweight, it does not necessarily describe the cardiometabolically critical fat content and distribution [140]. Alternative measures, particularly reflecting abdominal obesity (waist circumference, waist-to-hip ratio, waist-to-height ratio), have been shown to be more accurate risk predictors [140]. Respective subgroup analyses revealed, however, that the relation between birth weight and later overweight risk was even strengthened when non-BMI related measures were applied. Also subgroup analyses considering gestational age, age at follow-up, geographic origin etc. showed no significant influence on the overall outcome. Moreover, pooled adjusted estimates were calculated considering the influence of confounders on the strength of the relation under investigation. However, they had

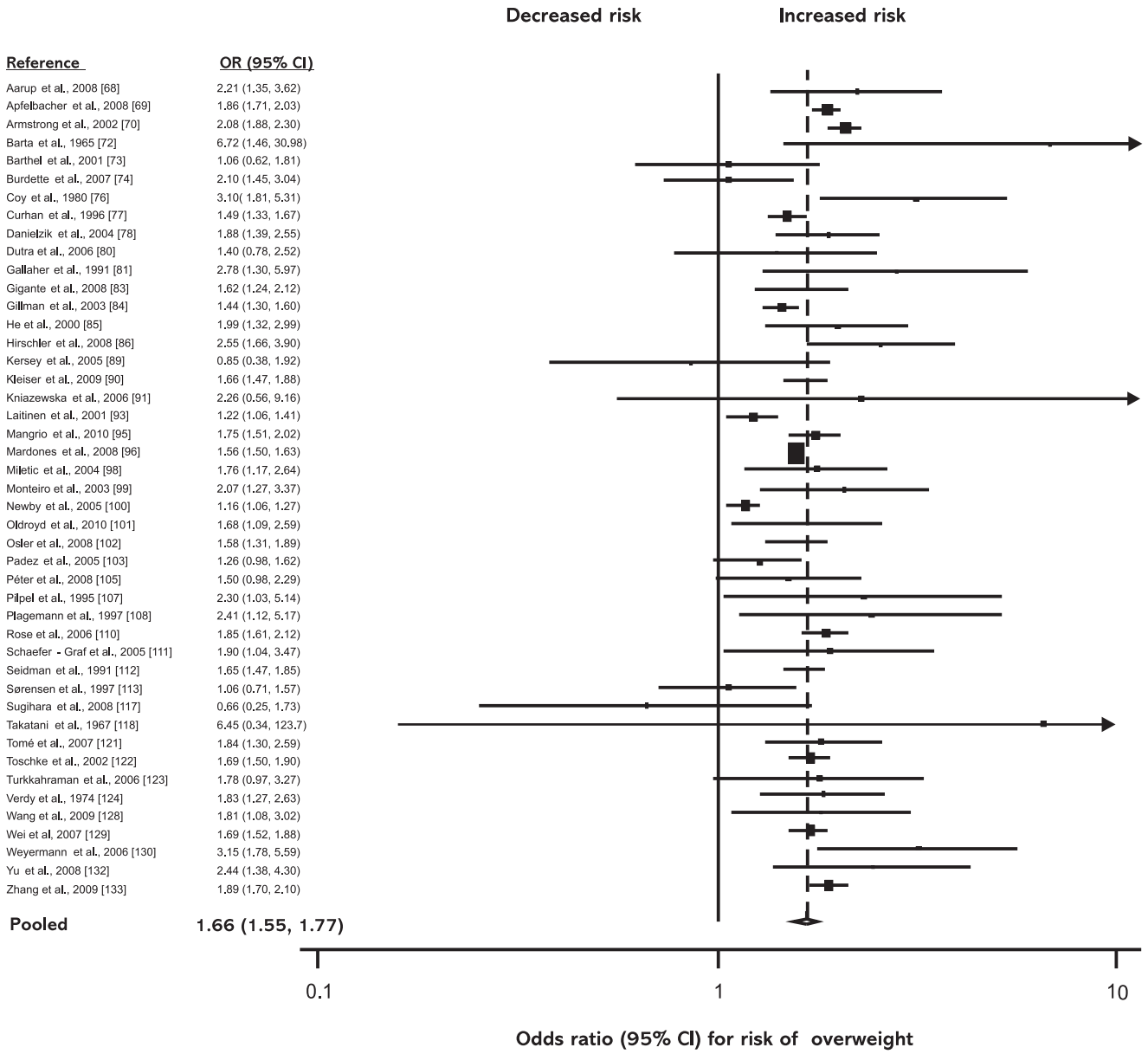

Figure 3. High birth weight (>4,000 g) and subsequent risk of overweight. ORs for overweight in subjects with birth weights >4,000 g as compared with subjects with birth weights ≤4,000 g. Studies are ordered alphabetically by first author. The point estimate *(center of each black square)* and the statistical size *(proportional area of square)* are represented. Horizontal lines indicate 95% confidence intervals. The pooled odds ratio *(diamond)* was calculated by means of a random effects model. OR, odds ratio; CI, confidence interval.
doi:10.1371/journal.pone.0047776.g003

no significant impact on the final outcome. Even parental weight status had only a marginal influence on the relation between birth weight and later overweight risk. This result appears to be of particular interest, since parental weight/overweight might represent contribution of genetic factors to the investigated relationship. Interestingly, it has been shown that none of the genetic obesity risk factors identified to date has a significant influence on birth weight [141], making respective confounding rather unlikely, as supported here.

Recently, Yu et al. [17] published a systematic review on the association between birth weight and downstream obesity, including 129,260 subjects from 33 studies, mainly originating from Asia (58%). Their meta-analysis involved 20 studies with a total of 42,863 subjects, most from China (15 studies; 88% of subjects). We analyzed a similar number of studies from Asia (n = 12) with a higher number of subjects from Asia (n = 140,734) as well as China (n = 103,411) in our meta-analysis. Interestingly, focussing on Asian/Chinese subjects Yu et al. [17] came to similar results as presented here: low birth weight was accompanied by decreased risk of obesity later on (OR = 0.61; 95% CI: 0.46–0.80), while high birth weight was associated with increased obesity risk (OR = 2.07; 95% CI: 1.91–2.24). Their analysis focused solely on obesity, with the meta-analysis only on children and adolescents including mainly Asian/Chinese subjects, studies and databases. A number of variables (*e.g.*, geographic origin/ethnicity, gestational age, age at follow-up, parental weight etc.) were not considered and confounder adjusted estimates were not provided. Nevertheless, the observed trend in the relation under investigation was

Table 1. Birth weight and later risk of overweight: sensitivity and confounder analyses*.

Category	Low birth weight Odds ratio (95% CI)	High birth weight Odds ratio (95% CI)
Number of studies	**n = 30**	**n = 45**
Study design		
cohort studies	0.67 (0.59−0.76) (n = 29)	1.66 (1.56−1.78) (n = 40)
case-control studies	0.17 (0.02−1.50) (n = 1)	2.05 (1.51−2.78) (n = 5)
Geographic origin		
Europe	0.72 (0.54−0.95) (n = 11)	1.63 (1.44−1.84) (n = 20)
North America	0.76 (0.61−0.95) (n = 4)	1.64 (1.43−1.88) (n = 7)
South America	0.53 (0.42−0.68) (n = 10)	1.69 (1.48−1.93) (n = 6)
Asia	0.83 (0.48−1.42) (n = 4)	1.75 (1.62−1.89) (n = 10)
Australia	0.50 (0.32−0.77) (n = 1)	2.23 (1.22−4.06) (n = 2)
Publication language		
English	0.67 (0.59−0.77) (n = 26)	1.68 (1.57−1.80) (n = 38)
Non English	0.63 (0.43−0.94) (n = 4)	1.60 (1.30−1.97) (n = 7)
Age at follow up		
0–18 years	0.60 (0.54−0.67) (n = 23)	1.76 (1.65−1.87) (n = 37)
>18 years	0.97 (0.79−1.20) (n = 7)	1.40 (1.23−1.59) (n = 8)
Overweight classification criterion		
BMI	0.68 (0.60−0.78) (n = 25)	1.63 (1.53−1.74) (n = 36)
Non BMI	0.52 (0.24−1.11) (n = 5)	2.26 (1.85−2.75) (n = 9)
Assessment of birth weight		
registry	0.74 (0.32−1.69) (n = 1)	1.39 (0.88−2.19) (n = 2)
records/examination	0.68 (0.57−0.81) (n = 14)	1.72 (1.56−1.89) (n = 22)
interview/questionnaire	0.65 (0.53−0.80) (n = 14)	1.65 (1.48−1.83) (n = 18)
not reported	0.17 (0.02−1.50) (n = 1)	2.08 (0.69−6.19) (n = 3)
Assessment of overweight		
records/examination	0.63 (0.56−0.71) (n = 26)	1.71 (1.61−1.82) (n = 39)
interview/questionnaire	0.88 (0.70−1.11) (n = 4)	1.45 (1.24−1.71) (n = 5)
not reported	-	6.72 (1.45−31.0) (n = 1)
Gender distribution		
≤50% males	0.59 (0.41−0.85) (n = 10)	1.65 (1.43−1.91) (n = 18)
>50% males	0.70 (0.62−0.78) (n = 18)	1.71 (1.60−1.83) (n = 23)
not reported	0.66 (0.52−0.84) (n = 2)	1.68 (1.55−1.82) (n = 4)
Lost-to-follow up		
≤20%	0.57 (0.46−0.70) (n = 11)	1.72 (1.58−1.88) (n = 13)
>20%	0.74 (0.61−0.89) (n = 17)	1.65 (1.49−1.83) (n = 26)
not reported	0.53 (0.23−1.22) (n = 2)	1.80 (1.60−2.02) (n = 6)
Parental SES		
low SES>30% of population	0.71 (0.60−0.83) (n = 5)	1.64 (1.47−1.18) (n = 7)
low SES≤30% of population	0.58 (0.52−0.65) (n = 5)	1.78 (1.63−1.94) (n = 9)
not reported	0.70 (0.58−0.85) (n = 20)	1.61 (1.45−1.79) (n = 29)
Gestational age		
only term newborns	0.59 (0.41−0.84) (n = 3)	1.67 (1.44−1.94) (n = 9)
term and preterm newborns	0.65 (0.57−0.74) (n = 6)	1.70 (1.46−1.97) (n = 5)
not reported	0.68 (0.56−0.82) (n = 21)	1.66 (1.51−1.82) (n = 31)
Parental overweight		
>30% of population	0.69 (0.41−1.18) (n = 2)	1.58 (1.29−1.94) (n = 6)
≤30% of population	-	1.73 (1.55−1.93) (n = 4)
not reported	0.67 (0.58−0.76) (n = 28)	1.66 (1.54−1.79) (n = 35)
Confounder-adjusted analyses		

Table 1. Cont.

Category	Low birth weight Odds ratio (95% CI)	High birth weight Odds ratio (95% CI)
Number of studies	**n = 30**	**n = 45**
Studies with adjustments		
unadjusted estimates	*0.50 (0.32−0.78) (n = 1)*	*1.87 (1.56−2.25) (n = 16)*
adjusted estimates	*0.51 (0.33−0.80) (n = 1)*	*1.93 (1.56−2.38) (n = 16)*
Studies without adjustments	*0.68 (0.60−0.77) (n = 29)*	*1.68 (1.54−1.84) (n = 29)*

Abbreviation: BMI, body mass index; CI, confidence interval
*random-effects model
doi:10.1371/journal.pone.0047776.t001

similar to those ascertained and described in our study. This appears to underline the global impact and reproducibility of the observed relationship and indicates that it is even relevant for Asian populations, characterized by a high frequency of relatively 'low birth weight' subjects but, simultaneously, continuous increase of 'diabesity' prevalence [142,143].

Therefore, it must be noted that the provided data analyses should not be interpreted in terms of a 'beneficial' effect of a reduced birth weight. To the contrary, epidemiological, clinical as well as experimental observations have convincingly demonstrated long-term deleterious consequences in association with a decreased birth weight, especially concerning metabolic-syndrome-like disorders and diseases [10,11]. Therefore, future studies should consider more precise measures of body composition, fat content and, especially, accompanying metabolic and hormonal alterations both at birth and later life to better understand pathophysiological links between altered prenatal nutritional and growth conditions and later overweight and 'diabesity' risk. Future studies should generally consider important variables in the relationship between fetal growth and later outcome, especially the potential impact of gestational age, maternal diseases during pregnancy, as well as neonatal nutrition, growth pattern and fat deposition. Finally, a slight trend has been observed here towards successively increasing risk of overweight in low birth weight subjects with increasing age, *i.e.*, in adulthood (Table 1). Therefore, a 'U-shaped' curve, as it has been described regarding type 2 diabetes and hypertension [136,134], with increasing age up to the elderly of the relation between birth weight and later risk of overweight, cannot be excluded from our data. Occasionally, pathophysiological causes and mechanisms of a latency of overweight manifestation in formerly low birth weight subjects remain to be evaluated.

Birth weight is essentially determined by the *in-utero* developmental conditions (13, 14), especially the materno-fetal food supply [144–146]. Accordingly, it appears important to notice that in parallel with the global 'diabesity' epidemics [2,3]; the number of overweight and/or diabetic women at reproductive age has increased dramatically [147,148]. Overweight and/or diabetes during pregnancy, however, lead to fetal overnutrition, often followed by increased birth weight, fatness and macrosomia at birth [144–146,149,150]. All of this has been shown to be preventable by adequate nutritional and metabolic management during pregnancy [151,152]. With respect to our data of increased

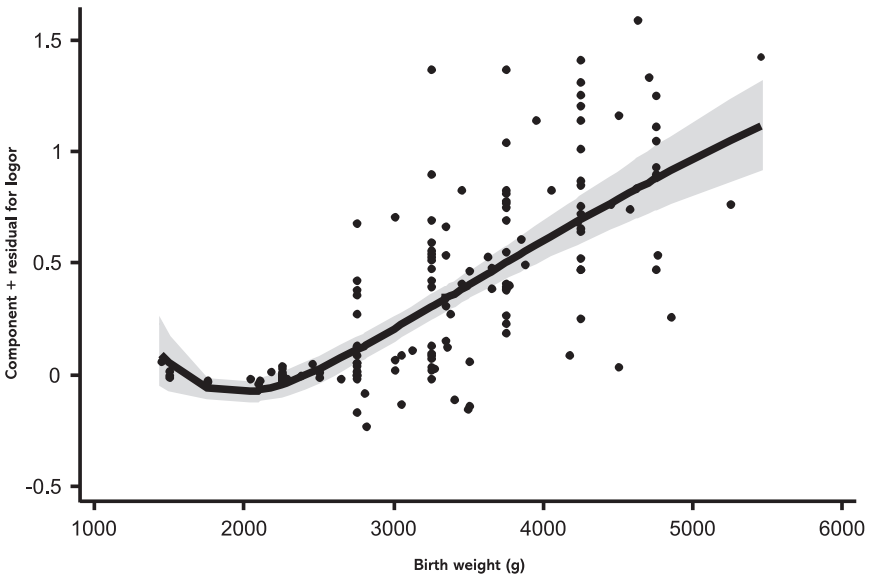

Figure 4. Relationship between birth weight and risk of overweight. Continuous relation between birth weight and later risk of overweight, calculated by fractional polynomial regression. Studies are represented by black dots. Grey shading indicates the 95% confidence interval around the fitted line. The model was estimated from a robust regression model based on second-order fractional polynomial (−1, −0.5) functions weighted by/variance.
doi:10.1371/journal.pone.0047776.g004

overweight risk in formerly macrosomic newborns, respective preventive measures may therefore not only improve the peripartal and perinatal outcome [151,152], but even the long-term overweight risk and resulting disease dispositions. This prediction is in line with a number of epidemiological, clinical and experimental data which have shown an increased risk of overweight, obesity and diabetes in offspring of diabetic and/or obese pregnant women, even independent of or in addition to genetic dispositions [153–157]. Especially, fetal hyperglycemia/overnutrition leads to fetal B cell hyperplasia and hyperinsulinism, which have been shown to be preserved for the long term and subsequently may predispose to insulin resistance and a permanent obesity disposition [144–146,158–161]. In general, mechanistic approaches speak in favour of epigenomic and/or microstructural long-term malprogramming of body weight regulatory systems by fetal overfeeding and accompanying hormonal disturbances during critical periods of fetal development, predisposing to increased overweight risk for the long-term [146,154,157–162].

Accordingly, prenatal life appears to be a 'critical period' [163,164] of determination and, consequently, potential genuine prevention of long-term overweight predisposition and its critical co-morbidity [165]. Interestingly, high birth weight has also been described to be a risk factor for, *e.g.*, type 2 diabetes, hypertension, childhood primary brain tumors and breast cancer [136,134,166,167], all shown to be critically linked to overweight throughout life [2–4,168].

Taken together, increased birth weight is reproducibly and independently linked to increased overweight risk later on, suggesting prenatal overfeeding as important risk factor which 'programms' a long-term obesity predisposition. In conclusion, avoiding *in-utero* overnutrition, especially by avoiding and/or adequately managing maternal overweight, overnutrition, increased weight gain and/or diabetes during pregnancy, appears to be a promising strategy to lower overweight risk for the long term, globally.

Supporting Information

Checklist S1 PRISMA checklist.
(DOC)

Protocol S1 Study protocol for systematic review and meta-analysis to determine the relation between birth weight and long-term overweight risk.
(DOC)

Table S1 Characteristics of 108 studies included in the systematic review of birth weight and subsequent risk of overweight, 1966–January 2011.
(DOC)

Table S2 Studies that adjusted for confounders in the meta-analysis on birth weight and subsequent risk of overweight, 1966-January 2011.
(DOC)

Table S3 Studies that reported data on neonatal weight gain or infant growth in the meta-analysis on birth weight and subsequent risk of overweight, 1966-January 2011.
(DOC)

Author Contributions

Conceived and designed the experiments: AP TH. Performed the experiments: KS SS . Analyzed the data: KS SS TH AP. Contributed reagents/materials/analysis tools: KS SS TH AP. Wrote the paper: KS SS TH AP.

References

1. King D (2011) The future challenge of obesity. Lancet 378: 743–744.
2. Mokdad AH, Ford ES, Bowman BA, Dietz WH, Vinicor F, et al. (2003) Prevalence of obesity, diabetes, and obesity related health risk factors, 2001. JAMA 289: 76–79.
3. Ogden CL, Carroll MD, Curtin LR, Lamb MM, Flegal KM (2010) Prevalence of high body mass index in US children and adolescents, 2007–2008. JAMA 303: 242–249.
4. Tirosh A, Shai I, Afek A, Dubnov-Raz G, Ayalon N, et al. (2011) Adolescent BMI trajectory and risk of diabetes versus coronary disease. N Engl J Med 364: 1315–1325.
5. Nordestgaard BG, Palmer TM, Benn M, Zacho J, Tybjaerg-Hansen A, et al. (2012) The effect of elevated body mass index on ischemic heart disease risk: causal estimates from a mendelian randomisation approach. PLoS Med 9: e1001212.
6. Prospective studies collaboration (2009) Body mass index and cause-specific mortality in 900000 adults: collaborative analyses of 57 prospective studies. Lancet 373: 1083–1096.
7. Berrington de Gonzalez A, Hartge P, Cerhan JR, Flint AJ, Hannan L, et al. (2010) Body-mass index and mortality among 1.46 million white adults. N Engl J Med 363: 2211–2219.
8. Franks PW, Hanson RL, Knowler WC, Sievers ML, Bennett PH, et al. (2010) Childhood obesity, other cardiovascular risk factors, and premature death. N Engl J Med 362: 485–493.
9. Boggs DA, Rosenberg L, Cozier YC, Wise LA, Coogan PF, et al. (2011) General and abdominal obesity and risk of death among black women. N Engl J Med 365: 901–908.
10. Barker DJ, Hales CN, Fall CHD, Osmond C, Phipps K, et al. (1993) Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia 36: 62–67.
11. Gluckman PD, Hanson MA, Cooper C, Thornburg KL (2008) Effect of in utero and early-life conditions on adult health and disease. N Engl J Med 359: 61–73.
12. Hales CN, Barker DJ (2001) The thrifty phenotype hypothesis. Br Med Bull 60: 5–20.
13. Brooks AA, Johnson MR, Steer PJ, Pawson ME, Abdalla HI (1995) Birth weight: nature or nurture? Early Hum Dev 42: 29–35.
14. Lunde A, Melve KK, Gjessing HK, Skjaerven R, Irgens LM (2007) Genetic and environmental influences on birth weight, birth length, head circumference, and gestational age by use of population-based parent-offspring data. Am J Epidemiol 165: 734–741.
15. Harder T, Schellong K, Stupin J, Dudenhausen JW, Plagemann A (2007) "Where is the evidence that low birthweight leads to obesity?" [letter]. Lancet 369: 1859.
16. Baird J, Fisher D, Lucas P, Kleijnen J, Roberts H, et al. (2005) Being big or growing fast: systematic review of size and growth in infancy and later obesity. BMJ 331: 929.
17. Yu ZB, Han SP, Zhu GZ, Zhu C, Wang XJ, et al. (2011) Birth weight and subsequent risk of obesity: a systematic review and meta-analysis. Obes Rev 12: 525–542.
18. Moher D, Liberati A, Tetzlaff J, Altman DG, The Prisma Group (2009) Preferred reporting items for systematic reviews and meta-analyses: The prisma statement. PLoS Med 7: 1006–1012.
19. Higgins JP, Thompson SG, Deeks JJ, Altman DG (2003) Measuring inconsistency in meta-analysis. BMJ 327: 557–560.
20. Berlin JA, Longnecker MP, Greenland S (1993) Meta-analysis of epidemiologic dose-response data. Epidemiology 4: 218–228.
21. Greenland S, Longnecker MP (1992) Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. Am J Epidemiol 135: 1301–1308.
22. Royston P, Ambler G, Sauerbrei W (1999) The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol 28: 964–974.
23. Harder T, Roepke K, Diller N, Stechling Y, Dudenhausen JW, et al. (2009) Birth weight, early weight gain, and subsequent risk of type 1 diabetes: systematic review and meta-analysis. Am J Epidemiol 169: 1428–1436.
24. Cardwell CR, Patterson CC (2009) Re: "Birth weight, early weight gain, and subsequent risk of type 1 diabetes" [letter]. Am J Epidemiol 170: 529–530.
25. Harder T, Plagemann A (2009) "Authors' reply: Birth weight, early weight gain and subsequent risk of type 1 diabetes: systematic review and meta-analysis" [letter]. Am J Epidemiol 170: 530–531.
26. Araújo CL, Hallal PC, Nader GA, Neutzling MB, deFátima Vieira M, et al. (2009) Effect of birth size and proportionality on BMI and skinfold thickness in early adolescence: prospective birth cohort study. Eur J Clin Nutr 63: 634–639.
27. Binkin NJ, Yip R, Fleshood L, Trowbridge FL (1988) Birth weight and childhood growth. Pediatrics 82: 828–834.
28. Boney CM, Verma A, Tucker R, Vohr BR (2005) Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. Pediatrics 115: 290–296.

29. Bouhours-Nouet N, Dufresne S, de Casson FB, Mathieu E, Douay O, et al. (2008) High birth weight and early postnatal weight gain protect obese children and adolescents from truncal adiposity and insulin resistance: metabolically healthy but obese subjects? Diabetes Care 31: 1031–1036.
30. Celi F, Bini V, De Giorgi G, Molinari D, Faraoni F, et al. (2003) Epidemiology of overweight and obesity among school children and adolescents in three provinces of central Italy, 1993–2001: study of potential influencing variables. Eur J Clin Nutr 57: 1045–1051.
31. Charney E, Goodman HC, McBride M, Lyon B, Pratt R (1976) Childhood antecedents of adult obesity. Do chubby infants become obese adults? N Engl J Med 295: 6–9.
32. da Costa Ribeiro I, Taddei JAAC, Colugnatti F (2003) Obesity among children attending elementary public schools in São Paulo, Brazil: a case-control study. Public Health Nutr 6: 659–663.
33. Drachler M de L, Macluf SP, Leite JC, Aerts DR, Giugliani ER, et al. (2003) Risk factors for overweight in children from Southern Brazil (Portuguese). Cad Saúde Pública 19: 1073–1081.
34. de Moraes SA, Beltrán Rosas J, Mondini L, Freitas IC (2006) Prevalence of overweight and obesity, and associated factors in school children from urban area in Chilpancingo, Guerrero, Mexico, 2004 (Portuguese). Cad Saúde Pública 22: 1289–1301.
35. Dieu HTT, Dibley MJ, Sibbritt D, Hanh TT (2007) Prevalence of overweight and obesity in preschool children and associated socio-demographic factors in Ho Chi Minh City, Vietnam. Int J Pediatr Obes 2: 40–50.
36. Dubois L, Girard M (2006) Early determinants of overweight at 4.5 years in a population-based longitudinal study. Int J Obes 30: 610–617.
37. Eriksson J, Forsén T, Tuomilehto J, Osmond C, Barker D (2001) Size at birth, childhood growth and obesity in adult life. Int J Obes 25: 735–740.
38. Frye C, Heinrich J (2003) Trends and predictors of overweight and obesity in East German children. Int J Obes 27: 963–969.
39. Fuiano N, Rapa A, Monzani A, Pietrobelli A, Diddi G, et al. (2008) Prevalence and risk factors for overweight and obesity in a population of Italian schoolchildren: a longitudinal study. J Endocrinol Invest 31: 979–984.
40. Guimarães LV, de Azevedo Barros MB, Martins MSAS, Duarte EC (2006) Factors associated with overweight in schoolchildren (Portuguese). Rev Nutr 19: 5–17.
41. Hack M, Schluchter M, Cartar L, Rahman M, Cuttler L, et al. (2003) Growth of very low birth weight infants to age 20 years. Pediatrics 112 e:30–38.
42. Hawkins SS, Cole TJ, Law C; Millennium Cohort Study Child Health Group (2009) An ecological systems approach to examining risk factors for early childhood overweight: findings from the UK Millennium Cohort Study. J Epidemiol Community Health 63: 147–155.
43. Hui LL, Nelson EA, Yu LM, Li AM, Fok TF (2003) Risk factors for childhood overweight in 6-7-y-old Hong Kong children. Int J Obes 27: 1411–1418.
44. Koupil I, Toivanen P (2008) Social and early-life determinants of overweight and obesity in 18-year-old Swedish men. Int J Obes (Lond) 32: 73–81.
45. Leong NM, Mignone LI, Newcomb PA, Titus-Ernstoff L, Baron JA, et al. (2003) Early life risk factors in cancer: the relation of birth weight to adult obesity. Int J Cancer 103: 789–791.
46. Li C, Goran MI, Kaur H, Nollen N, Ahluwalia JS (2007) Developmental trajectories of overweight during childhood: role of early life factors. Obesity 15: 760–771.
47. Locard E, Mamelle N, Billette A, Miginiac M, Munoz F, et al. (1992) Risk factors of obesity in a five year old population. Parental versus environmental factors. Int J Obes 16: 721–729.
48. Lundgren EM, Cnattingius S, Jonsson B, Tuvemo T (2003) Prediction of adult height and risk of overweight in females born small-for-gestational-age. Paediatr Perinat Epidemiol 17: 156–163.
49. Maddah M (2009) Risk factors for overweight in urban and rural school girls in Iran: skipping breakfast and early menarche. Int J Cardiol 136: 235–238.
50. Mazur A, Małecka-Tendera E, Klimek K (2003) Risk factors of obesity in primary school children from the Podkarpacie Region Part II. Obesity risk factors in girls (Polish). Pediatr Pol 78: 889–895.
51. Mazur A, Małecka-Tendera E, Klimek K (2003) Risk factors of obesity in primary school children from the Podkarpacie Region Part I. Obesity risk factors in boys. (Polish). Pediatr Pol 78: 881–888.
52. Mazur A, Klimek K, Telega G, Hejda G, Wdowiak L, et al. (2008) Risk factors for obesity development in school children from south-eastern Poland. Ann Agric Environ Med 15: 281–285.
53. Meas T, Deghmoun S, Armoogum P, Alberti C, Levy-Marchal C (2008) Consequences of being born small for gestational age on body composition: an 8-year follow-up study. J Clin Endocrinol Metab 93: 3804–3809.
54. Mogan J (1986) Parental weight and its relation to infant feeding patterns and infant obesity. Int J Nurs Stud 23: 255–264.
55. O'Callaghan MJ, Williams GM, Andersen MJ, Bor W, Najman JM (1997) Prediction of obesity in children at 5 years: a cohort study. J Paediatr Child Health 33: 311–316.
56. Ochoa MC, Moreno-Aliaga MJ, Martínez-González MAM, Martínez JA, Marti A; GENOI Members (2007) Predictor factors for childhood obesity in a Spanish case-control study. Nutrition 23: 379–384.
57. Olson CM, Strawderman MS, Dennison BA (2009) Maternal weight gain during pregnancy and child weight at age 3 years. Matern Child Health J 13: 839–846.
58. Rugholm S, Baker JL, Olsen LW, Schack-Nielsen L, Bua J, et al. (2005) Stability of the association between birth weight and childhood overweight during the development of the obesity epidemic. Obes Res 13: 2187–2194.
59. Savva SC, Tornaritis M, Chadjigeorgiou C, Kourides YA, Savva ME, et al. (2005) Prevalence and socio-demographic associations of undernutrition and obesity among preschool children in Cyprus. Eur J Clin Nutr 59: 1259–1265.
60. Seidman DS, Laor A, Stevenson DK, Sivan E, Gale R, et al. (1998) Macrosomia does not predict overweight in late adolescence in infants of diabetic mothers. Acta Obstet Gynecol Scand 77: 58–62.
61. Serra-Majem L, Bartrina JA, Pérez-Rodrigo C, Ribas-Barba L, Delgado-Rubio A (2006) Prevalence and determinants of obesity in Spanish children and young people. Br J Nutr 96: S67–72.
62. Shehadeh N, Weitzer-Kish H, Shamir R, Shihab R, Weiss R (2008) Impact of early postnatal weight gain and feeding patterns on body mass index in adolescence. J Pediatr Endocrinol Metab 21: 9–15.
63. Strufaldi MW, Silva EM, Franco MC, Puccini RF (2009) Blood pressure levels in childhood: probing the relative importance of birth weight and current size. Eur J Pediatr 168: 619–624.
64. Sugimori H, Yoshida K, Miyakawa M, Izuno T, Kishimoto T, et al. (1997) Influence of behavioral and environmental factors on the development of obesity in three-year-old children. A case-control study based on Toyama Study. Environ Health Prev Med 2: 74–78.
65. Takahashi E, Yoshida K, Sugimori H, Miyakawa M, Izuno T, et al. (1999) Influence factors on the development of obesity in 3-year-old children based on the Toyama study. Prev Med 28: 293–296.
66. Terry MB, Wei Y, Esserman D (2007) Maternal, birth, and early-life influences on adult body size in women. Am J Epidemiol 166: 5–13.
67. Vohr BR, Lipsitt LP, Oh W (1980) Somatic growth of children of diabetic mothers with reference to birth size. J Pediatr 97: 196–199.
68. Aarup M, Sokolowski I, Lous J (2008) The prevalence of obesity and overweight among 3 year-old children in the municipality of Aalborg and identification of risk factors (Danish). Ugeskr Laeger 170: 452–456.
69. Apfelbacher CJ, Loerbroks A, Cairns J, Behrendt H, Ring J, et al. (2008) Predictors of overweight and obesity in five to seven-year-old children in Germany: results from cross-sectional studies. BMC Public Health 8: 171.
70. Armstrong J, Reilly JJ, Child Health Information Team (2002) Breastfeeding and lowering the risk of childhood obesity. Lancet 359 2003–2004.
71. Barros AJ, Victora CG, Santos IS, Matijasevich A, Araújo Cl, et al. (2008) Infant malnutrition and obesity in three population-based birth cohort studies in Southern Brazil: trends and differences. Cad Saude Publica 24: S417–426.
72. Barta L, Domján O (1965) Birthweight of obese and diabetic children. Acta Paediatr Acad Sci Hung 6: 163–170.
73. Barthel B, Cariou C, Lebas-Saison E, Momas I (2001) Prevalence of obesity in childhood: study in Paris elementary schools (French). Santé Publique 13: 7–15.
74. Burdette HL, Whitaker RC (2007) Differences by race and ethnicity in the relationship between breastfeeding and obesity in preschool children. Ethn Dis 17: 467–470.
75. Chen A, Pennell ML, Klebanoff MA, Rogan WJ, Longnecker MP (2006) Maternal smoking during pregnancy in relation to child overweight: follow-up to age 8 years. Int J Epidemiol 35: 121–130.
76. Coy JF, Lewis IC, Mair CH, Longmore EA, Ratkowsky DA (1973) The growth of Tasmanian infants from birth to three years of age. Med J Aust 2: 12–18.
77. Curhan GC, Willett WC, Rimm EB, Spiegelman D, Ascherio AL, et al. (1996) Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. Circulation 94: 3246–3250.
78. Danielzik S, Czerwinski-Mast M, Langnäse K, Dilba B, Müller MJ (2004) Parental overweight, socioeconomic status and high birth weight are the major determinants of overweight and obesity in 5-7 y-old children: baseline data of the Kiel Obesity Prevention Study (KOPS). Int J Obes 28: 1494–1502.
79. Dennison BA, Edmunds LS, Stratton HH, Pruzek RM (2006) Rapid infant weight gain predicts childhood overweight. Obesity 14: 491–499.
80. Dutra CL, Araújo CL, Bertoldi AD (2006) Prevalence of overweight in adolescents: a population-based study in a southern Brazilian city (Portuguese). Cad Saúde Pública 22: 151–162.
81. Gallaher MM, Hauck FR, Yang-Oshida M, Serdula MK (1991) Obesity among mescalero preschool children. Association with maternal obesity and birth weight. Am J Dis Child 145:1262–1265.
82. Gigante DP, Victora CG, Araújo CLP, Barros FC (2003) Trends in the nutritional profile of children born in 1993 in Pelotas, Rio Grande do Sul, Brazil: Longitudinal analyses (Portuguese). Cad Saúde Pública 19: 141–147.
83. Gigante DP, Minten GC, Horta BL, Barros FC, Victora CG (2008) [Nutritional evaluation follow-up of the 1982 birth cohort, Pelotas, Southern Brazil] (Portuguese). Rev Saude Publica 42: 60–69.
84. Gillman MW, Rifas-Shiman S, Berkey CS, Field AE, Colditz GA (2003) Maternal gestational diabetes, birth weight, and adolescent obesity. Pediatrics 111: 221–226.
85. He Q, Ding ZY, Fong DY, Karlberg J (2000) Risk factors of obesity in preschool children in China: a population-based case-control study. Int J Obes 24: 1528–1536.
86. Hirschler V, Bugna J, Roque M, Gilligan T, Gonzalez C (2008) Does low birth weight predict obesity/overweight and metabolic syndrome in elementary school children? Arch Med Res 39:796–802.
87. Hui LL, Nelson EA, Yu LM, Li AM, Fok TF (2003) Risk factors for childhood overweight in 6–7-y-old Hong Kong children. Int J Obes 27: 1411–1418.

88. Kang HT, Ju YS, Park KH, Kwon YJ, Im HJ, et al. (2006) Study on the relationship between childhood obesity and various determinants, including socioeconomic factors, in an urban area (Korean). J Prev Med Pub Health 39: 371–378.
89. Kersey M, Lipton R, Sanchez-Rosado M, Kumar J, Thisted R (2005) Breast-feeding history and overweight in Latino preschoolers. Ambul Pediatr 5: 355–358.
90. Kleiser C, Schaffrath Rosario A, Mensink BM G, Urban K, Bukowska C (2009) Potential determinants of obesity among children and adolescents in Germany: results from the cross-sectional KiGGS study. BMC Public Health 9: 46.
91. Kniażewska M, Obuchowicz A, Żmudzińska-Kitczak J, Urban K, Bukowska C (2006) Assessment of association of birth weight and existence of hypertension in children and adolescents normostenic, obese or with metabolic syndrome. (Polish). Przegl Lek 63: 118–120.
92. Kromeyer-Hauschild K, Zellner K, Jaeger U, Hoyer H (1999) Prevalence of overweight and obesity among school children in Jena (Germany). Int J Obes 23: 1143–1150.
93. Laitinen J, Power C, Jarvelin MR (2001) Family social class, maternal body mass index, childhood body mass index, and age of menarche as predictors of adult obesity. Am J Clin Nutr 74:287–294.
94. Maffeis C, Micciolo R, Must A, Zaffanello M, Pinelli L (1994) Parental and perinatal factors associated with childhood obesity in north-east Italy. Int J Obes 18: 301–305.
95. Mangrio E, Lindström M, Rosvall M (2010) Early life factors and being overweight at 4 years of age among children in Malmö, Sweden. BMC Public Health 10: 764.
96. Mardones F, Villarroel L, Karzulovic L, Barja S, Arnaiz P (2008) Association of perinatal factors and obesity in 6- to 8-year-old Chilean children. Int J Epidemiol 37: 902–910.
97. Mikulandra F, Grgurić J, Banović I, Perisa M, Zakanj Z (2000) The effect of high birth weight (4000 g or more) on weight and height of adult men and women. Coll Antropol 24: 133–136.
98. Miletić T, Mikulandra F, Stoini E, Anic J, Perisa M (2004) The relationship of birth weight and length to weight, height and body mass index in adult women. (Croatian). Gynaecol Perinatol 13: 13–5.
99. Monteiro POA, Victora CG, Barros FC, Monteiro LMA (2003) Birth size, early childhood growth, and adolescent obesity in a Brazilian birth cohort. Int J Obes 27: 1274–1282.
100. Newby PK, Dickman PW, Adami HO, Wolk A (2005) Early anthropometric measures and reproductive factors as predictors of body mass index and obesity among older women. Int J Obes 29: 1084–1092.
101. Oldroyd J, Renzaho A, Skouteris H (2010) Low and high birth weight as risk factors for obesity among 4 to 5-year-old Australian children: does gender matter? Eur J Pediatr 170: 899–906.
102. Osler M, Lund R, Kriegbaum M, Andersen AM (2009) The influence of birth weight and body mass in early adulthood on early coronary heart disease risk among Danish men born in 1953. Eur J Epidemiol 24: 57–61.
103. Padez C, Mourão I, Moreira P, Rosado V (2005) Prevalence and risk factors for overweight and obesity in Portuguese children. Acta Paediatr Int J Paediatr 94: 1550–1557.
104. Panagiotakos DB, Papadimitriou A, Anthracopoulos MB, Konstantinidou M, Antonogeorgos G (2008) Birthweight, breast-feeding, parental weight and prevalence of obesity in schoolchildren aged 10-12 years, in Greece; the Physical Activity, Nutrition and Allergies in Children Examined in Athens (PANACEA) study. Pediatr Int 50: 563–568.
105. Péter S, Bíró L, Németh A, Antal M (2008) Association between birth weight and childhood obesity in a Budapest metropolitan survey. (Hungarian). Orv Hetil. 149: 407-410.
106. Phillips DIW, Young JB (2000) Birth weight, climate at birth and the risk of obesity in adult life. Int J Obes 24: 281–287.
107. Pilpel D, Leer A, Phillip M (1995) Obesity among Jewish and Bedouin secondary school students in the Negev, Israel. Public Health Rev 23: 253–262.
108. Plagemann A, Harder T, Kohlhoff R, Rohde W, Dörner G (1997) Overweight and obesity in infants of mothers with long-term insulin-dependent diabetes or gestational diabetes. Int J Obes 21: 451–456.
109. Reilly JJ, Armstrong J, Dorosty AR, Emmett PM, Ness A (2005) Early life risk factors for obesity in childhood: cohort study. BMJ 330: 1357.
110. Rose D, Bodor JN (2006) Household food insecurity and overweight status in young school children: Results from the Early Childhood Longitudinal study. Pediatrics 117: 464–473.
111. Schaefer-Graf UM, Pawliczak J, Passow D, Hartmann R, Rossi R (2005) Birth weight and parental BMI predict overweight in children from mothers with gestational diabetes. Diabetes Care. 28: 1745–1750.
112. Seidman DS, Laor A, Gale R, Stevenson DK, Danon YL (1991) A longitudinal study of birth weight and being overweight in late adolescence. Am J Dis Child 145: 782–785.
113. Sørensen HT, Sabroe S, Rothman KJ, Gillman M, Fischer P, et al. (1997) Relation between weight and length at birth and body mass index in young adulthood: cohort study. BMJ 315: 1137.
114. Stettler N, Bovet P, Shamlaye H, Zemel BS, Stallings VA, et al. (2002) Prevalence and risk factors for overweight and obesity in children from Seychelles, a country in rapid transition: the importance of early growth. Int J Obes 26: 214–219.
115. Stettler N, Zemel BS, Kumanyika S, Stallings VA (2002) Infant weight gain and childhood overweight status in a multicenter, cohort study. Pediatrics 109: 194–199.
116. Stettler N, Kumanyika SK, Katz SH, Zemel BS, Stallings VA (2003) Rapid weight gain during infancy and obesity in young adulthood in a cohort of African Americans. Am J Clin Nutr 77: 1374–1378.
117. Sugihara S, Sasaki N, Amemiya S, Kohno H, Tanaka T (2008) Analysis of weight at birth and at diagnosis of childhood-onset type 2 diabetes mellitus in Japan. Pediatr Diabetes 9: 285–290.
118. Takatani H, Okada T, Kaneda A (1967) Survey of infant obesity 3 years later. (Japanese). Nippon Shonika Gakkai Zasshi 71: 309–313.
119. Tene CE, Espinoza-Mejía MY, Silva-Rosales NA, Girón-Carrillo JL (2003) High birth weight as a risk factor for childhood obesity. (Spanish). Gac Med Mex 139: 15–20.
120. Tian JY, Cheng Q, Song XM, Li G, Jiang GX (2006) Birth weight and risk of type 2 diabetes, abdominal obesity and hypertension among Chinese adults. Eur J Endocrinol 155: 601–607.
121. Tomé FS, Cardoso VC, Barbieri MA, Silva AA, Simões VM (2007) Are birth weight and maternal smoking during pregnancy associated with malnutrition and excess weight among school age children? Braz J Med Biol Res 40: 1221–1230.
122. Toschke AM, Vignerova J, Lhotska L, Osancova K, Koletzko B, et al. (2002) Overweight and obesity in 6- to 14-year-old Czech children in 1991: protective effect of breast-feeding. J Pediatr 141: 764–769.
123. Turkkahraman D, Bircan I, Tosun O, Saka O (2006) Prevalence and risk factors of obesity in school children in Antalya, Turkey. Saudi Med J 27: 1028–1033.
124. Verdy M, Gagnon MA, Caron D (1974) Birth weight and adult obesity in children of diabetic mothers. N Engl J Med 290: 576.
125. Vitolo MR, Gama CM, Bortolini GA, Campagnolo PD, Drachler Mde L (2008) Some risk factors associated with overweight, stunting and wasting among children under 5 years old. J Pediatr (Rio J) 84: 251–257.
126. von Kries R, Koletzko B, Sauerwald T, von Mutius E, Barnert D, et al. (1999) Breast feeding and obesity: cross sectional study. BMJ 319: 147–150.
127. von Kries R, Toschke AM, Koletzko B, Slikker W (2002) Maternal smoking during pregnancy and childhood obesity. Am J Epidemiol 156: 954–961.
128. Wang Y, Gao E, Wu J, Zhou J, Yang Q, et al. (2009) Fetal macrosomia and adolescence obesity: results from a longitudinal cohort study. Int J Obes 33: 923–928.
129. Wei JN, Li HY, Sung FC, Lin CC, Chiang CC, et al. (2007) Birth weight correlates differently with cardiovascular risk factors in youth. Obesity 15: 1609–1616.
130. Weyermann M, Rothenbacher D, Brenner H (2006) Duration of breastfeeding and risk of overweight in childhood: a prospective birth cohort study from Germany. Int J Obes 30: 1281–1287.
131. Ylihärsilä H, Kajantie E, Osmond C, Forsén T, Barker DJ, et al. (2007) Birth size, adult body composition and muscle strength in later life. Int J Obes 31: 1392–1399.
132. Yu Z, Sun JQ, Haas JD, Gu Y, Li Z, et al. (2008) Macrosomia is associated with high weight-for-height in children aged 1–3 years in Shanghai, China. Int J Obes 32: 55–60.
133. Zhang X, Liu E, Tian Z, Wang W, Ye T, et al. (2009) High birth weight and overweight of obesity among Chinese children 3.6 years old. Preventive Medicine 49: 172–178.
134. Huxley R, Neil A, Collins R (2002) Unravelling the fetal origins hypothesis: is there really an inverse association between birthweight and subsequent blood pressure? Lancet 360: 659–665.
135. Huxley R, Owen CG, Whincup PH, Cook DG, Colman S, et al. (2004) Birth weight and subsequent cholesterol levels. Exploration of the "fetal origins" hypothesis. JAMA 292: 2755–2764.
136. Harder T, Rodekamp E, Schellong K, Dudenhausen JW, Plagemann A (2007) Birth weight and subsequent risk of type 2 diabetes: a meta-analysis. Am J Epidemiol 165: 849–857.
137. Whincup PH, Kaye SJ, Owen CG, Huxley R, Cook DG, et al. (2008) Birth weight and risk of type 2 diabetes. A systematic review. JAMA 300: 2886–2897.
138. Plagemann A, Harder T (2009) Re: "Birth weight and risk of type 2 diabetes in adults" [letter]. JAMA 301: 1540–1541.
139. Bhargava SK, Sachdev HS, Fall CHD, Osmond C, Lakshmy R, et al. (2004) Relation of serial changes in childhood body-mass index to impaired glucose tolerance in young adulthood. N Engl J Med 350: 865–875.
140. Lee CMY, Huxley RR, Wildman RP, Woodward M (2008) Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: a meta-analysis. J Clin Epidemiol 61: 646–653.
141. Kilpeläinen TO, den Hoed M, Ong KK, Grøntved A, Brage S, et al. (2011) Obesity-susceptility loci have a limited influence on birth weight: a meta-analysis of up to 28,219 individuals. Am J Clin Nutr 93: 851–860.
142. Hossain P, Kawar B, El Nahas M (2007) Obesity and diabetes in the developing world - a growing challenge. N Engl J Med 356: 213–215.
143. Scully T (2012) Diabetes in numbers. Nature 485: S2–3.
144. Freinkel N, Metzger BE (1979) Pregnancy as a tissue culture experience: the critical implications of maternal metabolism for fetal development. In: Pregnancy metabolism, diabetes, and the fetus. Ciba Foundation Symposium 63. Amsterdam: Excerpta Medica. pp 3–28.
145. Freinkel N (1980) Of pregnancy and progeny. Diabetes 29: 1023–1035.

146. Catalano P, Haughel-De Mouzon S (2011) Is it time to revisit the Pedersen hypothesis in the face of the obesity epidemic? Am J Obstet Gynecol 204: 479–487.
147. Jovanovic L, Pettitt DJ (2001) Gestational diabetes mellitus. JAMA 286: 2516–2518.
148. The HAPO study collaborative research group (2008) Hyperglycemia and adverse pregnancy outcomes. N Engl J Med 358: 1991–2002.
149. Cedergren MI (2004) Maternal morbid obesity and the risk of adverse pregnancy outcome. Obstet Gynecol 103: 219–224.
150. Sewell MF, Huston-Presley L, Super DM, Catalano P (2006) Increased neonatal fat mass, not lean body mass, is associated with maternal obesity. Am J Obstet Gynecol 195: 1100–1003.
151. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, et al. (2005) Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. N Engl J Med 352: 2477–2486.
152. Landon MB, Spong CY, Thom E, Carpenter MW, Ramin SM, et al. (2009) A multicenter, randomized trial of treatment for mild gestational diabetes. N Engl J Med 361: 1339–1348.
153. Pettitt DJ, Baird HR, Aleck KA, Bennett PH, Knowler WC (1983) Excessive obesity in offspring of Pima Indian women with diabetes during pregnancy. N Engl J Med 308: 242–245.
154. Silverman BL, Metzger BE, Cho NH, Loeb CA (1995) Impaired glucose tolerance in adolescents offspring of diabetic mothers. Relationship to fetal hyperinsulinism. Diabetes Care 18: 611–617.
155. Plagemann A, Harder T, Kohlhoff R, Rohde W, Dörner G (1997) Overweight and obesity in infants of mothers with long-term insulin-dependent diabetes or gestational diabetes. Int J Obes Relat Metab Disord 21: 451–456.
156. Nelson SM, Matthews P, Poston L (2010) Maternal metabolism and obesity: modifiable determinants of pregnancy outcome. Hum Reprod Update 16: 255–275.
157. Levin BE (2010) Developmental gene x environment interactions affecting systems regulating energy homeostasis and obesity. Front Neuroendocrinol 31: 270–283.
158. Dörner G, Plagemann A (1994) Perinatal hyperinsulinism as possible predisposing factor for diabetes mellitus, obesity and enhanced cardiovascular risk in later life. Horm Metab Res 26: 213–221.
159. Plagemann A (2008) A matter of insulin: Developmental programming of body weight regulation. J Mat Fet Med 21: 143–148.
160. Van Assche FA, Holemans K, Aerts L (2001) Long-term consequences for offspring of diabetes during pregnancy. Br Med Bull 60: 173–182.
161. Van Assche FA, Devlieger R, Harder T, Plagemann A (2010) Mitogenic effect of insulin and developmental programming. Diabetologia 53: 1243.
162. Holemans K, Aerts L, Van Assche FA (1991) Evidence for an insulin resistance in the adult offspring of pregnant streptozotocin-diabetic rats. Diabetologia 34: 81–85.
163. Stockard CR (1921) Developmental rate and structural expression: an experimental study of twins, ''double monsters'' and single deformities, and the interaction among embryonic organs during their origin and development. Am J Anat 28: 115–263.
164. Tzschentke B, Plagemann A (2006) Imprinting and critical periods in early development. World's Poult Sci J 62: 627–638.
165. Plagemann A, Harder T, Dudenhausen JW (2010) Re: ''Childhood obesity, other cardiovascular risk factors, and premature death'' [letter]. N Engl J Med 362: 1840–1841.
166. Harder T, Plagemann A, Harder A (2008) Birth weight and subsequent risk of childhood primary brain tumors: A meta-analysis. Am J Epidemiol 168: 366–373.
167. Silva Idos S, De Stavola B, McCormack V; Collaborative Group on Pre-Natal Risk Factors and Subsequent Risk of Breast Cancer. (2008) Birth size and breast cancer risk: re-analysis of individual participant data from 32 studies. PLoS Med 5: e193.
168. Ehemann C, Henly SJ, Ballard-Barbash R, Jacobs EJ, Schymura MJ, et al. (2012) Annual report to the nation on the status of cancer, 1975–2008, featuring cancers associated with excess weight and lack of sufficient physical activity. Cancer 118: 2338–2366.

Neuroscience Letters 515 (2012) 87–91

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Short-term regulation of the hypothalamic melanocortinergic system under fasting and defined glucose-refeeding conditions in rats: A lasercapture microdissection (LMD)-based study

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ARTICLE INFO

Article history:
Received 30 January 2012
Received in revised form 27 February 2012
Accepted 9 March 2012

Keywords:
Hypothalamus
Proopiomelanocortin
Agouti-related peptide
Fasting
Glucose-refeeding
Lasercapture microdissection

ABSTRACT

It is well established that under fasting conditions the expression of the orexigenic neuropeptide agouti-related peptide (AGRP) is up-regulated in the hypothalamic arcuate nucleus (ARC), while inconsistent data exist regarding fasting regulation of the anorexigenic neurohormone proopiomelanocortin (POMC). Inconsistencies might have methodological reasons, especially concerning neuromorphological and/or experimental (nutritional) specificity. We analyzed the expression of both neuropeptides in ARC neurons, using lasercapture microdissection (LMD) and real-time PCR in 12 h fasted *vs.* fed Wistar rats as well as after a standardized glucose load, *i.e.*, under clinically relevant conditions in terms of diagnosing glucose intolerance in the human. Under fasting conditions, clear up-regulation of AGRP was observed, with increasing magnitude in ARC single neurons (SNP) as compared to ARC cell layers (+125% *vs.* +23%, resp.), closely correlated to hypoinsulinemia and hypoleptinemia. Surprisingly, in the fasting state POMC was not found to be down-regulated, neither in ARC cell layers nor in ARC single neurons (+9% *vs.* +6%). However, glucose-refeeding under diagnostically relevant conditions led to strong neuronal up-regulation of POMC expression in ARC SNP (+128%), and AGRP down-regulation (−50%). In conclusion, experimentally, topographically, and analytically specific and standardized conditions confirmed AGRP in ARC neurons as being neuronally up- and down-regulated, resp., depending on the general nutritional state, while POMC was found to be (up-) regulated only after peripheral glucose load. Findings suggest that POMC in ARC neurons acts glucose-mediated as an "anti-orexigenic" neurohormone, specifically responding to hyperglycemia.

1. Introduction

Food intake and body weight are regulated by a complex system of peripheral and central signals. Within the hypothalamic melanocortinergic system proopiomelanocortin (POMC) and agouti-related peptide (AGRP) exert opposing effects on food intake and energy balance [35]. POMC, the precursor molecule of the anorexigenic post-translational cleavage peptide α-melanocyte-stimulating hormone (α-MSH), is synthesized in the hypothalamus exclusively in the arcuate hypothalamic nucleus (ARC) [11]. *Via* axonal α-MSH transport into the paraventricular nucleus (PVN) and activation of the melanocortin-4 receptor (MC4-R) there, an increase in POMC expression in the ARC leads to reduced food intake. On the opposite, orexigenic AGRP, which is also expressed exclusively in the ARC [14,23], functions as endogenous antagonist [24] and inverse agonist [16] at melanocortin receptors. Thus, central administration of AGRP results in a stimulation of food intake [36]. Within this regulatory system, leptin and insulin act as circulating satiety signals from the periphery. POMC and AGRP neurons co-express leptin and insulin receptors [3,7]. Physiologically, circulating leptin and insulin are considered to stimulate the expression of POMC, while inhibiting expression of AGRP [4,17,34].

A considerable proportion of the data that led to the above-mentioned regulatory paradigm came from studies in fasting *vs.* fed animals. It is well established that during fasting the expression of AGRP is increased, due to decreased levels of plasma leptin and insulin, as demonstrated in the whole hypothalamus [21,25] as well as specifically in the ARC [6,32]. However, regarding POMC regulation in the fasting state the existing literature is inconsistent. Some studies observed a significant down-regulation of POMC expression in whole hypothalamus samples [5,21,22,37] and in the ARC [1,6], while other studies reported no POMC regulation,

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doi:10.1016/j.neulet.2012.03.025

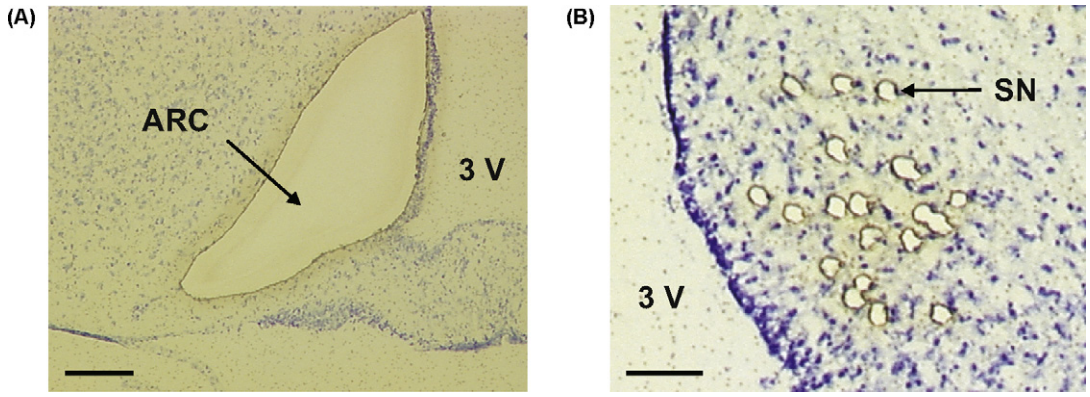

Fig. 1. Lasercapture microdissection of the arcuate nucleus (ARC) from rat hypothalamus. Cell layer preparation of the whole ARC ((A) scale bar = 100 μm), and single neuron preparations from the contralateral ARC ((B) scale bar = 50 μm), isolated from 10 μm-Nissl stained cryosections. 3 V = third ventricle; SN = microdissected single neuron.

neither after short-term fasting [25] nor after longer fasting periods [30]. Some of these discrepancies may arise from different fasting regimens used (24 h *vs.* 48 h *vs.* 72 h), combined with non-specific approaches to re-feed animals. Additionally, differences in neuroanatomical specificity (whole hypothalamus *vs.* whole ARC *vs.* single ARC neurons) might be relevant.

In recent years lasercapture microdissection (LMD) has become a powerful technique to obtain small samples and/or even specific cell populations in heterogeneous tissues such as the brain [13,38]. We therefore analyzed the expression of AGRP and POMC in ARC neurons, using LMD combined with subsequent real-time PCR after a fasting period of 12 h, which corresponds to clinically relevant regimens as in glucose tolerance tests. Accordingly, further standardization was achieved by a specific refeeding (standardized glucose load), instead of providing *ad libitum* feeding which leads to a mixed macronutrient effect.

2. Materials and methods

Experiments were performed in male Wistar rats, weighing 400–450 g, of an outbred colony strain (Charles River, Sulzfeld, Germany). Animals were kept under standard conditions with 12 h:12 h inverse light–dark rhythm and free access to tap water and standard pellet diet (control diet for rats, ssniff, Soest, Germany, Code V1536-000). All animal procedures were in accordance with the European Communities Council Directive (86/609/EEC) and approved by the local animal welfare committee (G 0026/07; Lageso Berlin, Germany). One group of rats was fed *ad libitum* (fed, $n = 4$), another group was studied after fasting for 12 h (12 h fasted, $n = 7$). A third group received 0.75 ml of a 20% glucose solution/100 g body weight (*i.e.*, 1.5 g/kg) intraperitoneally after 12 h fasting (12 h fasted + glucose, $n = 3$). 30 min after glucose injection rats were rapidly decapitated. All animals were sacrificed in the morning between 08.00 and 09.00 a.m.

Trunk blood was collected for determination of blood glucose, immunoreactive plasma insulin and leptin. Glucose was measured photometrically (glucoseoxidase-peroxidase method; Dr. Lange GmbH, Berlin, Germany). For determination of insulin and leptin, commercial radioimmunoassays were used (Linco, St. Charles, MO, USA). Recombinant rat insulin and leptin (Linco) served as standard preparation. The intra-assay variation was 1.4–4.6% in a concentration range of 0.5–3.7 ng/ml for insulin ($n = 14$), and 2.4–4.6% in a concentration range of 1.6–11.6 ng/ml ($n = 14$) for leptin [15,28].

Following rapid decapitation, brains were quickly removed, frozen in isopentane and stored at −70 °C. For LMD, 10 μm-thick coronary serial sections were cut through the deep-frozen hypothalami, mounted on glass slides (Leica frame slides with 1.4 μm Polyethylene terephthalat (PET)-membrane), dried on air, and finally Nissl-stained with cresyl violet under RNase-free conditions. Staining method was chosen to cover standards of ensuring neuronal specificity, allowing data comparability with other studies [26], and to avoid detection bias in between-group comparisons in subsequent mRNA-analyses. After staining, slides were kept at −70 °C until LMD. To verify anatomical location of the ARC, a rat brain atlas was used [27]. Samples were captured by Leica Microsystems AS/LMD® instrument (Leica Microsystems CMS GmbH, Wetzlar, Germany) across the full rostral-caudal extent of the ARC, corresponding to levels 26–32 as defined by Paxinos and Watson [27]. In each of the serial sections, the following procedure was carried out: On the left-hand side of the hypothalamus, the ARC was prepared as a whole cell layer (Fig. 1A). Then, from the contralateral, *i.e.*, right-hand side of same sections, in total 100 neurons were picked individually and pooled (single neuron pools, SNP) (Fig. 1B). To ensure neuronal specificity, only neurons with a distinct nucleolus and soma appearance were LMD-prepared and used for subsequent measurements [15]. Both microdissection as well as subsequent analyses were performed in a blinded manner by two independent investigators (E.L., K.S.).

Total RNA was extracted from samples and DNase treated using the PureLink RNA Micro Kit (Invitrogen) according to the manufacturer's instructions. The RNA was dissolved in 14 μl RNase-free water and samples were stored at −70 °C until further processing. 7.5 μl RNA was reverse-transcribed into complementary DNA (cDNA) using Superscript-First-Strand-Synthesis System (Invitrogen, Carlsbad, USA), and 2 μl cDNA were amplified in subsequent real-time PCR.

Duplex real-time PCR was performed in triplicate in an Applied Biosystems 7500 instrument [29]. POMC and AGRP mRNA expression were analyzed using commercial intron-spanning TaqMan® gene expression assays from Applied Biosystems (Rn00595020_m1, FAM-labeled for POMC, and Rn01431703_g1, FAM-labeled for AGRP), together with an endogenous control assay for the housekeeping gene beta Actin (4352340E, VIC-labeled). Standard protocol conditions consisted of denaturation at 95 °C for 10 min, followed by 40 two-step cycles at 95 °C for 15 s and 60 °C for 1 min (TaqMan Gene Expression Assays Protocol, part number 4333458, Applied Biosystems). mRNA quantification referred to the $2^{-\Delta Ct}$ method [33]. For determination of PCR efficiency (E), we examined a serial dilution of target- and housekeeping gene and calculated $E = 10^{(-1/\text{slope})}$. Relative expression of target genes (*vs.* beta Actin) was determined by respective Ct according to the equation [31]:

$$\frac{E_{\text{target gene}}^{-\text{Ct(target gene)}}}{E_{\text{beta Actin}}^{-\text{Ct(beta Actin)}}}$$

Table 1
Metabolic parameters.

Parameter	Fed ($n=4$)	12 h fasted ($n=7$)	12 h fasted + glucose ($n=3$)
Blood glucose (mmol/l)	5.26 ± 0.35	4.36 ± 0.14*	10.34 ± 1.33#
Insulin (ng/ml)	3.55 ± 0.36	1.01 ± 0.31*	6.50 ± 1.80#
Leptin (ng/ml)	4.55 ± 0.64	0.91 ± 0.24*	6.44 ± 3.62#

Values are expressed as means ± SEM. Number of animals in parenthesis.
* $p < 0.05$ *vs.* fed by Mann–Whitney *U*-test.
# $p < 0.05$ *vs.*12 h fasted by Mann–Whitney *U*-test.

Data are expressed as means ± SEM. Real-time PCR data are given as arbitrary units. Mann–Whitney *U*-test was used to analyze group differences. For analysis of relations between two variables, Spearman's rank correlation test was performed (SPSS Software 18.0, Munich, Germany).

3. Results

As an experimental precondition [9], we first verified that mRNA expression of the housekeeping gene beta Actin was unaffected by respective feeding conditions. Neither fasting nor glucose-refeeding led to changes of beta Actin mRNA expression in ARC cell layers or ARC SNP's (data not shown).

12 h fasting led to a decrease in plasma concentrations of glucose (−17%), insulin (−72%) and leptin (−80%) (Table 1). Hypoleptinemia and hypoinsulinemia were associated with a significant increase of AGRP expression in ARC cell layers (+23%; fed *vs.* 12 h fasted: 0.26 ± 0.01 *vs.* 0.32 ± 0.01; $p \leq 0.05$), while POMC expression did not differ significantly between groups (fed *vs.* 12 h fasted: 1.45 ± 0.20 *vs.* 1.59 ± 0.20; n.s.). Measurement in pools of 100 single ARC neurons revealed an increase in AGRP expression in fasted *vs.* fed animals of even +125% (fed *vs.* 12 h fasted: 0.16 ± 0.03 *vs.* 0.36 ± 0.02; $p \leq 0.05$). Again, however, POMC expression showed no significant group difference (fed *vs.* 12 h fasted: 1.49 ± 0.22 *vs.* 1.58 ± 0.16; n.s.) (Fig. 2A).

Rats that received a glucose load after 12 h fasting showed strongly increased plasma glucose (+137%), insulin (+544%) and leptin (+608%) (Table 1). Accordingly, in ARC cell layers AGRP expression was found to be down-regulated and did no longer differ between fasted and glucose-refed group (12 h fasted *vs.* 12 h fasted + glucose: 0.19 ± 0.02 *vs.* 0.20 ± 0.02; n.s.). Moreover, in single neuron pools AGRP expression was even found to be significantly decreased in 12 h fasted + glucose rats (12 h fasted *vs.* 12 h fasted + glucose: 0.10 ± 0.01 *vs.* 0.05 ± 0.02; $p \leq 0.05$).

This was accompanied by a significant up-regulation of POMC expression in ARC cell layers of +44% (12 h fasted *vs.* 12 h fasted + glucose: 0.94 ± 0.01 *vs.* 1.35 ± 0.08; $p \leq 0.05$). In ARC single neurons, a POMC expression increase of even +128% was observed (12 h fasted *vs.* 12 h fasted + glucose: 0.57 ± 0.05 *vs.* 1.30 ± 0.21; $p \leq 0.05$) (Fig. 2B).

4. Discussion

Although it is an established paradigm that POMC and AGRP act within the hypothalamic melanocortinergic system as antagonists in the regulation of food intake and body weight, controversial data exist on whether and how POMC expression is regulated in response to starvation.

After 12 h fasting no significant regulation of POMC expression was detected here, neither in ARC cell layers nor in ARC single neurons (Fig. 2A). Similar was observed by investigating whole hypothalamus in a number of other studies. Neither short-term fasting of 4 h or 8 h nor long-term fasting periods of 48 h or 72 h led to a change of POMC expression in the whole hypothalamus, measured by real-time- or RT-PCR [19,25,30]. Even in the distinct ARC no significant regulation of POMC expression after a 24 h-fasting period was detected by *in situ* hybridization [8]. This is in contrast to reports of a significant decrease of POMC expression measured by real-time PCR in the whole hypothalamus, or POMC suppression determined by Northern Blot in the whole ARC [20,22].

As demonstrated here, application of single neuron LMD gives no indication for POMC regulation under 12 h fasting conditions. This observation in ARC single neurons was underpinned by missing correlations of POMC mRNA to insulin ($r = -0.05$; $p = 0.91$) and leptin ($r = 0.38$; $p = 0.35$). In parallel, however, expression of the orexigenic neuropeptide AGRP was significantly increased after 12 h fasting, confirming a number of other studies [6,12,21,25,32,37]. Worthy to note, in the only LMD-based study so far [26], which was performed in diet-induced obese rats exposed to an energy-dense high-fat diet, POMC was observed to be decreased by only 22% after a 4-times longer fasting period of

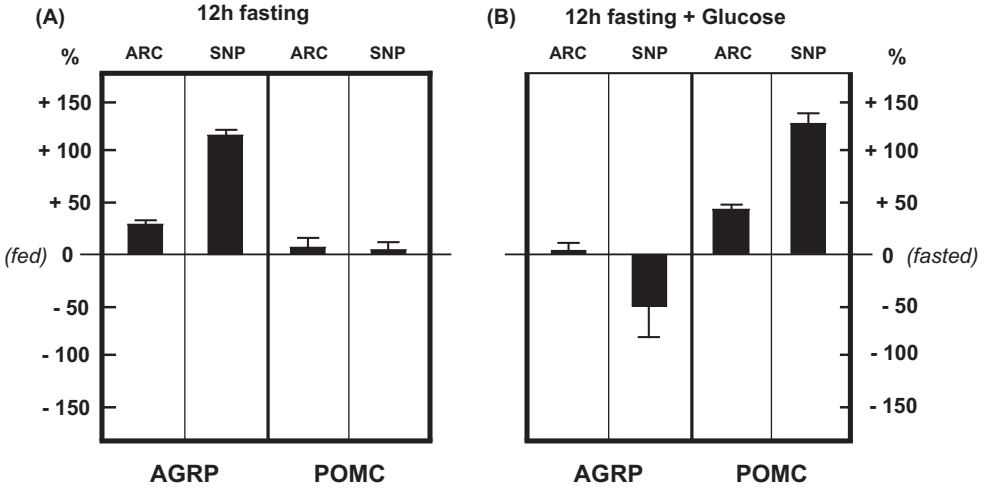

Fig. 2. mRNA expression of agouti-related peptide (AGRP) and proopiomelanocortin (POMC) within cell layers of the arcuate hypothalamic nucleus (ARC) as compared to single neuron pools ($n = 100$ neurons per animal) from the ARC (SNP) under different fasting and glucose-refeeding regimens. (A) Data show that fasting ($n = 4$ animals), compared to *ad libitum* feeding ($n = 7$ animals), leads to an increase in AGRP expression, with increasing magnitude in ARC SNP as compared to ARC cell layers, whereas POMC expression is not changed. (B) Rather, POMC expression increases due to a standardized peripheral glucose load ($n = 3$ animals) as compared to fasted animals. Relative responses are presented as percentage of respective controls (±SEM).

48 h, as compared to respectively high-caloric fed rats. AGRP was simultaneously increased by 113%. Accordingly, our data show that fasting for only 12 h in normal rats under normal dietary conditions, leads to an increase in AGRP expression in ARC single neurons of +125% as compared to +23% in whole ARC cell layers (Fig. 2A). Data validity in ARC SNP's is supported by inverse correlations of AGRP expression to insulin ($r = -0.79$; $p = 0.02$) and leptin ($r = -0.69$; $p = 0.09$).

Consequently, the question arose of a specific nutritional regulation of POMC expression. In fact, this could be demonstrated here through a standardized glucose-refeeding. Fasting followed by a defined peripheral glucose load led to a significant up-regulation of POMC mRNA, with increasing measurable magnitude of POMC expression from ARC cell layers (+44%) to ARC SNP's (+128%) (Fig. 2B). In parallel, AGRP expression was significantly decreased in single neuron pools (−50%), confirming the expected counter-regulation of the orexigenic system in this nutritional state. Accordingly, with increasing specificity of the investigated material (ARC cell layers *vs.* ARC single neurons), expression of AGRP in fasted animals as well as POMC expression in the glucose-refed group was gradually found to be up-regulated, thereby strongly supporting the sensitivity of the used method (LMD combined with real-time PCR).

Concerns might be raised according to the 'one dose–one time point' approach of our study. However, it should be noted that pioneer studies in the field used very similar approaches [2,10], finally leading to the establishment of the widely accepted insulin- and leptin-dependent regulation paradigm. Moreover, our study primarily addressed a translational, potentially clinically relevant approach ('glucose tolerance test' conditions). This might be of particular relevance for comparatively estimating respective basic research data. While our observations remain to be confirmed, of course, appropriate consideration of glucose levels in future studies, experimentally as well as clinically, might contribute to a more precise understanding of the regulations under investigation.

Interpreted integratively, data suggest that POMC expression in ARC neurons is not regulated under short-term fasting conditions (12 h), but responds highly sensitive and immediately (30 min) to hyperglycemia. This suggestion seems to be supported by the fact that 3.5 fold difference in insulin and 4.5 fold difference in leptin levels in fed *vs.* fasted rats were not accompanied by differences in POMC expression, while only 1.5 fold glucose increase gave rise to a 2.3 fold increase of POMC expression in ARC neurons (Table 1 and Fig. 2).

Summarizing, in addition to responding to insulin and leptin, hypothalamic POMC expression seems to depend on the peripheral, circulating glucose level as an additional regulator. Interestingly, while electrophysiological glucose responsiveness of POMC-expressing ARC neurons has been documented [18], in our study a significant correlation of POMC expression in ARC SNP to circulating, peripheral glucose levels was observed under hyperglycemic conditions ($r = 0.87$; $p = 0.02$). This speaks in favor of a direct response of the hypothalamic melanocortin system to postprandial changes in peripheral blood glucose levels. Most noteworthy, while intracerebroventricular glucose application did not significantly affect ARC POMC expression [12], a peripheral glucose load does so, as demonstrated here. Therefore, the influence of circulating glucose levels on hypothalamic POMC expression should be considered as important variable in future studies.

Even from a teleological point of view, the observed data and interpretation of a glucose-mediated regulation of hypothalamic POMC expression appears to be plausible. While acute, short-term activation of the POMC-driven HPA system fundamentally aims to mobilize endogenous glucose in parallel with inhibiting food intake under stressful conditions, a specifically glucose-mediated "anti-orexigenic" function of POMC expression in hypothalamic feeding circuits would logically fit with the overall stress-coping function of the 'POMC system', in general. Of course, this hypothesis remains to be verified.

In conclusion, the methodology described and proved here (neuronal LMD with subsequent real-time PCR) allows a highly precise and complex analysis of neuropeptidergic regulatory mechanisms of body weight and food intake in respective paradigms, potentially combining neuromorphological and neurogenetic analyses intraindividually, which is technically not feasible in more traditional approaches such as *in situ* hybridization. Our observations indicate a glucose-dependent regulation of hypothalamic POMC expression in a dose-response manner, specifically in response to hyperglycemia. This might have considerable implications for further investigations, interpretations and pharmacological considerations regarding functioning of the hypothalamic melanocortinergic system in the regulation of food intake and metabolism.

Acknowledgments

This study has been supported by the Deutsche Forschungsgemeinschaft (DFG; PL 6/1) and the Danone Institute Nutrition for Health, Germany (2011-1).

References

[1] R.S. Ahima, J. Kelly, J.K. Elmquist, J.S. Flier, Distinct physiologic and neuronal responses to decreased leptin and mild hyperleptinaemia, Endocrinology 140 (1999) 4923–4931.
[2] D.G. Baskin, J.F. Breininger, M.W. Schwartz, Leptin receptor mRNA identifies a subpopulation of neuropeptide Y neurons activated by fasting in rat hypothalamus, Diabetes 48 (1999) 828–833.
[3] D.G. Baskin, B.J. Wilcox, D.P. Figlewicz, D.M. Dorsa, Insulin and insulin-like growth factors in the CNS, Trends Neurosci. 11 (1988) 107–111.
[4] S.C. Benoit, E.L. Air, L.M. Coolen, R. Strauss, A. Jackman, D.J. Clegg, R.J. Seeley, S.C. Woods, The catabolic action of insulin in the brain is mediated by melanocortins, J. Neurosci. 22 (2002) 9048–9052.
[5] F. Bertile, H. Oudart, F. Criscuolo, Y.L. Maho, T. Raclot, Hypothalamic gene expression in long-term fasted rats: relationship with body fat, Biochem. Biophys. Res. Commun. 303 (2003) 1106–1113.
[6] S. Bi, B.M. Robinson, T.H. Moran, Acute food deprivation and chronic food restriction differentially affect hypothalamic NPY mRNA expression, Am. J. Physiol. Regul. Integr. Comp. Physiol. 285 (2003) R1030–R1036.
[7] C.C. Cheung, R.J. Clifton, R.A. Steiner, Proopiomelanocortin neurons are direct targets for leptin in the hypothalamus, Endocrinology 138 (1997) 4489–4492.
[8] M.F. Dallman, S.F. Akana, S. Bhatnagar, M.E. Bell, S. Choi, A. Chu, C. Horsley, N. Levin, O. Meijer, L.R. Soriano, A.M. Strack, V. Viau, Starvation: early signals, sensors, and sequelae, Endocrinology 140 (1999) 4015–4023.
[9] N.M. Derks, M. Müller, B. Gaszner, D.T.W.M. Tilburg-Ouwens, E.W. Roubos, L.T. Kozicz, Housekeeping genes revisited: different expressions depending on gender, brain area and stressor, Neuroscience 156 (2008) 305–309.
[10] J.K. Elmquist, R.S. Ahima, E. Maratos-Flier, J.S. Flier, C.B. Saper, Leptin activates neurons in ventrobasal hypothalamus and brainstem, Endocrinology 138 (1997) 839–842.
[11] K.L.J. Ellacott, R.D. Cone, The central melanocortin system and the integration of short- and long-term regulators of energy homeostasis, Recent Prog. Horm. Res. 59 (2004) 395–408.
[12] C. Fekete, P.S. Singru, E. Sanchez, S. Sarkar, M.A. Christoffolete, R.S. Riberio, W.M. Rand, C.H. Emerson, A.C. Bianco, R.M. Lechan, Differential effects of central leptin, insulin, or glucose administration during fasting on the hypothalamic–pituitary–thyroid axis and feeding-related neurons in the arcuate nucleus, Endocrinology 147 (2006) 520–529.
[13] S. Frank, E. Copanaki, G.J. Burbach, U.C. Müller, T. Deller, Differential regulation of toll-like receptor mRNAs in amyloid plaque-associated brain tissue of aged APP23 trangenic mice, Neurosci. Lett. 453 (2009) 41–44.
[14] T.M. Hahn, J.F. Breininger, D.G. Baskin, M.W. Schwartz, Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons, Nat. Neurosci. 1 (1998) 271–272.
[15] T. Harder, K. Franke, S. Fahrenkrog, L. Aerts, R. Van Bree, F.A. Van Assche, A. Plagemann, Prevention by maternal pancreatic islet transplantation of hypothalamic malformation in offspring of diabetic mother rats is already detectable at weaning, Neurosci. Lett. 352 (2003) 163–166.
[16] C. Haskell-Luevano, E.K. Monck, Agouti-related protein functions as an inverse agonist at a constitutively active brain melanocortin-4 receptor, Regul. Peptide 99 (2001) 1–7.
[17] T.L. Horvath, The hardship of obesity: a soft-wired hypothalamus, Nat. Neurosci. 8 (2005) 561–565.

[18] N. Ibrahim, M.A. Bosch, J.L. Smart, J. Qiu, M. Rubinstein, O.K. Rønnekleiv, M.J. Low, M.J. Kelly, Hypothalamic proopiomelanocortin neurons are glucose responsive and express K_{ATP} channels, Endocrinology 144 (2003) 1331–1340.
[19] L. Kappeler, P. Zizzari, D. Grouselle, J. Epelbaum, M.T. Bluet-Pajot, Plasma and hypothalamic peptide-hormone levels regulating somatotroph function and energy balance in fed and fasted states: a comparative study in four strains of rats, J. Neuroendocrinol. 16 (2004) 980–988.
[20] E.M. Kim, C.C. Welch, M.K. Grace, C.J. Billington, A.S. Levine, Chronic food restriction and acute food deprivation decrease mRNA levels of opioid peptides in arcuate nucleus, Am. J. Physiol. 270 (1995) R1019–R1024.
[21] J. Korner, E. Savontaus, J.S.C. Chua, R.L. Leibel, S.L. Wardlaw, Leptin regulation of AGRP and NPY mRNA in the rat hypothalamus, J. Neuroendocrinol. 13 (2001) 959–966.
[22] S. Kumano, H. Matsumoto, Y. Takatsu, J. Noguchi, C. Kitada, T. Ohtaki, Changes in hypothalamic expression levels of galanin-like peptide in rat and mouse models support that it is a leptin-target peptide, Endocrinology 144 (2003) 2634–2643.
[23] T.M. Mizuno, H. Makimura, C.V. Mobbs, The physiological function of the agouti-related peptide gene: the control of weight and metabolic rate, Ann. Med. 35 (2003) 425–433.
[24] M.M. Ollmann, B.D. Wilson, Y.K. Yang, J.A. Kerns, Y. Chen, I. Gantz, G.S. Barsh, Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein, Science 278 (1997) 135–138.
[25] M. Palou, J. Sánchez, A.M. Rodriguez, T. Priego, C. Picó, A. Palou, Induction of NPY/AgRP orexigenic peptide expression in rat hypothalamus is an early event in fasting: relationship with circulating leptin, insulin and glucose, Cell. Physiol. Biochem. 23 (2009) 115–124.
[26] S.J. Paulsen, L.K. Larsen, J. Jelsing, U. Janßen, B. Gerstmayer, N. Vrang, Gene expression profiling of individual hypothalamic nuclei from single animals using laser capture microdissection and microarrays, J. Neurosci. Methods 177 (2009) 87–93.
[27] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, 2nd ed., Academic Press, San Diego, 1986.
[28] A. Plagemann, T. Harder, A. Rake, T. Waas, K. Melchior, T. Ziska, W. Rohde, G. Dörner, Observations on the orexigenic hypothalamic neuropeptide Y-system in neonatally overfed weanling rats, J. Neuroendocrinol. 11 (1999) 541–546.
[29] A. Plagemann, K. Roepke, T. Harder, M. Brunn, A. Harder, M. Wittrock-Staar, T. Ziska, K. Schellong, E. Rodekamp, K. Melchior, J.W. Dudenhausen, Epigenetic malprogramming of the insulin receptor promoter due to developmental overfeeding, J. Perinat. Med. 38 (2010) 393–400.
[30] L.E. Pritchard, R.L. Oliver, J.D. McLoughlin, S. Birtles, C.B. Lawrence, A.V. Turnbull, A. White, Proopiomelanocortin-derived peptides in rat cerebrospinal fluid and hypothalamic extracts: evidence that secretion is regulated with respect to energy balance, Endocrinology 144 (2003) 760–766.
[31] H. Prüss, K. Prass, L. Gharni, M. Milosevic, C. Muselmann, D. Freyer, G. Royl, U. Reuter, N. Baeva, U. Dirnagl, A. Meisel, J. Priller, Inducible nitric oxide synthase does not mediate brain damage after transient focal cerebral ischemia in mice, J. Cereb. Blood Flow Metab. 28 (2008) 526–539.
[32] E. Sánchez, P.S. Singru, R. Acharya, M. Bodria, C. Fekete, A. Zavacki, A.C. Bianco, R.M. Lechan, Differential effects of refeeding on melanocortin-responsive neurons in the hypothalamic paraventricular nucleus, Endocrinology 149 (2008) 4329–4335.
[33] T. Schmittgen, K. Livak, Analyzing real-time PCR data by the comparative C_t method, Nat. Protocol 3 (2008) 1101–1108.
[34] M.W. Schwartz, R.J. Seeley, D.S. Weigle, L.A. Campfield, D.G. Baskin, Leptin increases hypothalamic proopiomelanocortin (POMC) mRNA expression in the rostral arcuate nucleus, Diabetes 46 (1997) 2119–2123.
[35] M.W. Schwartz, S.C. Woods, D.J. Porte, R.J. Seeley, D.G. Baskin, Central nervous system control of food intake, Nature 404 (2000) 661–671.
[36] C.J. Small, M.S. Kim, S.A. Stanley, J.R.D. Mitchell, K. Murphy, D.G.A. Morgan, M.A. Ghatei, S.R. Bloom, Effects of chronic central administration of agouti-related protein in pair-fed animals, Diabetes 50 (2001) 248–554.
[37] E. Sucajtys-Szulc, J. Turyn, E. Goyke, J. Korczynska, E. Stelmanska, E. Slominska, R.T. Smolenski, B. Rutkowski, J. Swierczynski, Differential effect of prolonged food restriction and fasting on hypothalamic malonyl-CoA concentration and expression of orexigenic and anorexigenic neuropeptides genes in rats, Neuropeptides 44 (2010) 17–23.
[38] W.Z. Wang, F.M. Oeschger, S. Lee, Z. Molnár, High quality RNA from multiple brain regions simultaneously acquired by laser capture microdissection, BMC Mol. Biol. 10 (2009) 69.

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Increase of Long-Term 'Diabesity' Risk, Hyperphagia, and Altered Hypothalamic Neuropeptide Expression in Neonatally Overnourished 'Small-For-Gestational-Age' (SGA) Rats

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Abstract

Background: Epidemiological data have shown long-term health adversity in low birth weight subjects, especially concerning the metabolic syndrome and 'diabesity' risk. Alterations in adult food intake have been suggested to be causally involved. Responsible mechanisms remain unclear.

Methods and Findings: By rearing in normal (NL) *vs.* small litters (SL), small-for-gestational-age (SGA) rats were neonatally exposed to either normal (SGA-in-NL) or over-feeding (SGA-in-SL), and followed up into late adult age as compared to normally reared appropriate-for-gestational-age control rats (AGA-in-NL). SGA-in-SL rats displayed rapid neonatal weight gain within one week after birth, while SGA-in-NL growth caught up only at juvenile age (day 60), as compared to AGA-in-NL controls. In adulthood, an increase in lipids, leptin, insulin, insulin/glucose-ratio (all $p<0.05$), and hyperphagia under normal chow as well as high-energy/high-fat diet, modelling modern 'westernized' lifestyle, were observed only in SGA-in-SL as compared to both SGA-in-NL and AGA-in-NL rats ($p<0.05$). Lasercapture microdissection (LMD)-based neuropeptide expression analyses in single neuron pools of the arcuate hypothalamic nucleus (ARC) revealed a significant shift towards down-regulation of the anorexigenic melanocortinergic system (*proopiomelanocortin*, *Pomc*) in SGA-in-SL rats ($p<0.05$). Neuropeptide expression within the orexigenic system (*neuropeptide Y* (*Npy*), *agouti-related-peptide* (*Agrp*) and *galanin* (*Gal*)) was not significantly altered. In essence, the 'orexigenic index', proposed here as a neuroendocrine 'net-indicator', was increased in SGA-in-SL regarding *Npy/Pomc* expression ($p<0.01$), correlated to food intake ($p<0.05$).

Conclusion: Adult SGA rats developed increased 'diabesity' risk only if exposed to neonatal overfeeding. Hypothalamic malprogramming towards decreased anorexigenic activity was involved into the pathophysiology of this neonatally acquired adverse phenotype. Neonatal overfeeding appears to be a critical long-term risk factor in 'small-for-gestational-age babies'.

Citation: Schellong K, Neumann U, Rancourt RC, Plagemann A (2013) Increase of Long-Term 'Diabesity' Risk, Hyperphagia, and Altered Hypothalamic Neuropeptide Expression in Neonatally Overnourished 'Small-For-Gestational-Age' (SGA) Rats. PLoS ONE 8(11): e78799. doi:10.1371/journal.pone.0078799

Editor: Miguel López, University of Santiago de Compostela School of Medicine - CIMUS, Spain

Received June 14, 2013; **Accepted** September 17, 2013; **Published** November 12, 2013

Funding: Aspects of this study were supported by grants of the German Research Foundation (DFG: 241/3, 241/4, 241/6-1). No additional external funding received for this study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Prevalence of obesity, diabetes and accompanying disturbances has increased globally reaching epidemic levels in adults, adolescents and even children [1–4]. To prevent the further spread of this epidemic, identifying early risk factors is urgently needed to develop appropriate prevention strategies.

Since the early 1990s, great attention has been given to the association between a low birth weight (LBW) and long-term risk of developing cardiovascular diseases, type 2 diabetes and the metabolic syndrome. The respective 'small-baby-syndrome' hypothesis proposes that poor materno-fetal nutrition leads to growth restriction and, consequently, long-lasting programming towards 'diabesogenic' alterations [5,6]. A 'thrifty phenotype' acquired *in utero* through poor fetal nutrition should enable affected individuals to better adaptation towards reduced food availability in later life [5,7]. However, when those individuals are exposed to affluent conditions later on, according to the hypothesis this acquired disposition leads to the development of type 2 diabetes, cardiovascular diseases, and the metabolic syndrome.

Many epidemiological studies have confirmed the phenomenological association between low birth weight and later development of symptoms of the metabolic syndrome [8–10]. Causal mechanisms, however, of the 'small-baby-syndrome' are still unclear. A recent epidemiological study showed that in formerly 'small babies', altered dietary habits are linked to the increased risk in later life [11], in line with the 'thrifty phenotype' hypothesis. Being small at birth was associated with higher intake of fat at later adult age.

However, a number of studies have reported that individuals born with high birth weight, induced by prenatal overnutrition, are also at increased risk of 'diabesity' later on. Meta-analyses demonstrated that both low and high birth weight are associated with increased risk of developing type 2 diabetes and hypertension [12–14]. Moreover, long-term risk for overweight, *i.e.*, the most important cardio-metabolic risk factor, has even been shown to be linearly *positively* related to birth weight [15]. The explanation of this developmental paradox remains unclear and the role of prenatal undernutrition and/or low birth weight as *independent* risk factors for the development of 'diabesity' and metabolic syndrome later on has to be challenged [16].

A number of animal studies, especially in rodents, were performed to investigate mechanisms of the association between reduced materno-fetal food supply, low birth weight (LBW) and later diseases. Rats with LBW, however, showed rather reduced body weight in the long-term, reduced food intake and normal glucose tolerance [17–19]. The fact that findings from animal models do not completely coincide with the observations from epidemiological studies leads to the suggestion that there must be additional factors predisposing to increased 'diabesity' risk in later life of 'small babies' [16].

Over several years, our group has proposed that neonatal overnutrition after low birth weight might play a decisive role in this scenario [20]. Currently, catch-up growth through 'rapid' neonatal weight gain has become a potential mechanism within the 'small-baby-syndrome' hypothesis [21–23]. To investigate consequences of neonatal overfeeding, *i.e.*, the probably most important cause of rapid neonatal weight gain, the small litter model is a long-established experimental paradigm [24–28]. Reduction of litter size in rodents causes increased weight gain during the early postnatal period due to qualitative as well as quantitative overnutrition [29]. Rats raised in small litters display early overweight, increased food intake, impaired glucose tolerance, hyperinsulinemia, hyperleptinemia, and hypertriglyceridemia in later life [25–27,30,31]. This neonatally acquired phenotype has been linked with permanent dysregulation of neuropeptides critically involved in the central nervous regulation of food intake and body weight [26,27,31–38].

Interestingly, altered food intake in the long-term has recently also been considered causal for adverse health outcomes in low birth weight humans [11]. Consequently, the question arises whether the increased risk of 'diabesogenic' alterations after low birth weight might rather be a consequence of neonatal overnutrition than fetal underfeeding and low birth weight *per se*. Up to now, this has rarely been considered in clinical and experimental studies.

Thus, we established a new, 'genuine' rat model of low birth weight to investigate the long-term outcome of 'small-for-gestational-age' rats additionally exposed to neonatal overnutrition, as compared to normal neonatal feeding [39]. We examined later food intake both under normal conditions by feeding standard laboratory chow as well as under dietary provocation by exposing the animals to a high-energy/high-fat diet at higher adult age. Long-term metabolic profile was characterized and hypothalamic expression patterns of orexigenic (*Agrp*, *Gal*, *Npy*) and anorexigenic (*Pomc*) neuropeptides in single neurons from the arcuate hypothalamic nucleus (ARC) were measured, using lasercapture microdissection (LMD) combined with quantitative real-time PCR to ensure highest possible specificity and sensitivity [40].

Materials and Methods

Ethics Statement

All animal procedures were carried out in accordance with the European Communities Council Directive (86/609/EEC) and were approved by the local animal welfare committee (G 0093/02; Lageso Berlin, Germany).

Animal Model and Study Design

Virgin female Wistar rats (Charles River Laboratories, Sulzfeld, Germany), weighing 200–250 g, were time mated with normal males and delivered spontaneously. Pups were defined as small-for-gestational-age (SGA) if their birth weight was below the lower limit of the 95% confidence interval of the mean birth weight of all pups of the same litter and sex. Pups which had a birth weight within the limits of the 95% confidence interval for litter and sex were assigned as appropriate-for-gestational-age (AGA). The study groups (neonatal overnutrition *vs.* control) were generated by adjusting the litter sizes *per* mother on day 3 of life into litters of only three pups (small litters, SL) or 12 pups (normal litters, NL) through random distribution [24,32]. SGA rats were raised then in normal (SGA-in-NL) or small litters (SGA-in-SL) until weaning. AGA rats were raised in normal litters, *i.e.*, under normal neonatal feeding conditions, and served as controls (AGA-in-NL).

After weaning (day 21 of life), female rats were housed under standard conditions with 12/12 h inverse light-dark rhythm, controlled temperature (22±2°C) and free access to tap water and standard laboratory chow (commercial control diet for rats; ssniff R/M-H, Soest, Germany, Code V1536–000). Feeding studies were performed from day 470 to 560 (see below). At day 560, animals were sacrificed and tissues and blood were collected.

Body Weight and Body Composition

Body weight and body length (nose to anus length) and mortality were monitored and recorded throughout life. Relative body weight/body length was evaluated in g/cm. On day 560, body composition was determined by weighing first the carcass mass after stomach and intestine removal. Next, dry mass and fat-free dry mass (FFDM) were determined by drying carcasses to constant weight followed by whole body chloroform extraction in a Soxhlet apparatus. FFDM and body fat were calculated as percentage of carcass mass [41].

Basal Metabolic Parameters

Blood samples were taken after an overnight fast (16 h) by puncture of the retroorbital plexus under light ether anaesthesia [31] at days 360 and 560 of life to determine basal metabolic parameters. Blood glucose was measured photometrically using the glucoseoxidase-peroxidase (GOD-PAP) method (Dr Lange GmbH, Berlin, Germany). Total plasma cholesterol and plasma triglyceride concentrations were quantified using the cholesterinoxidase-peroxidase (CHOD-PAP) method and the glyceride-3-phosphatoxidase-peroxidase (GPO-PAP) method, respectively (Dr Lange GmbH, Berlin, Germany).

Leptin concentration was quantified using a commercial radioimmunoassay (rat leptin RIA kit, Linco, St. Charles, MO, USA). Recombinant rat leptin (Linco) served as the standard preparation. The intra-assay variation ranged between 2.4–4.6% in a concentration range of 1.6–11.6 μg/l.

For determination of insulin, within one assay a modified commercial radioimmunoassay was performed (Adaltis, Freiburg, Germany). Rat insulin (Novo Nordisk Biolabs, Copenhagen, Denmark) with a biological potency of 21.3 IU/mg was used as standard preparation. The intra-assay coefficient of variation was

4.5–7.4% in a concentration range of 9.2–94.2 mIU/l. The insulin/glucose-ratio was calculated as a measure of peripheral insulin resistance [42].

Glucose Tolerance Test

Glucose tolerance tests were performed at days 130 and 530 of life. After an overnight period of fasting (16 h), a 20% glucose solution (1.5 g/kg body weight) was injected intraperitoneally. Blood samples were taken at 0, 15, 30, and 90 minutes after glucose loading for determination of blood glucose levels. Using these values, the area under the curve of glucose (AUCG) against time was calculated for each animal [31].

Food Intake Study

Food intake was studied at older adult age (between days 470 and 560 of life), with individual housing. First, food intake of standard laboratory chow was measured for 30 days (days 470–500 of life). Chow comprised of 9% fat, 33% protein, and 58% carbohydrates with a metabolizable energy content of 3.1 kcal/g (ssniff R/M-H, Soest, Germany, Code V1536-000). For the following 60 days (500–560 day of life), rats were exposed to a palatable high-energy/high-fat (HE/HF) diet containing 34% fat, 23% protein, 43% carbohydrates with a metabolizable energy content of 4.1 kcal/g (specific diet, Code 132006; Altromin, Lage, Germany). This was a modified version of the diet described by Levin et al. and has previously been shown as highly palatable [34,43]. As both diets have different energy contents caloric intake per day was calculated (kcal/d). Rats were fed *ad libitum* throughout the study period and had free access to tap water. Food intake was recorded daily and body weight measured weekly to the nearest 0.1 g (Sartorius MC 1, Laboratory LC 6200, Sartorius AG, Göttingen, Germany).

Neuropeptide Expression in the Hypothalamic Arcuate Nucleus (ARC)

Lasercapture Microdissection (LMD). Following rapid sacrifice on day 560 of life, brains were immediately isolated, frozen in isopentane and stored at −80°C. For LMD, 10 µm-thick coronary serial sections were cut through the deep-frozen hypothalami, mounted on glass slides (Leica frame slides with 1.4 µm Polyethylene terephthalat (PET)-membrane), dried on air, and finally Nissl-stained with cresyl violet under RNase-free conditions. After staining, slides were kept at −80°C until LMD. Anatomical location of the ARC was verified according to a rat brain atlas [44]. Using the Leica Microsystems AS/LMD® instrument (Leica Microsystems CMS GmbH, Wetzlar, Germany), in total 100 neurons were randomly picked individually from each brain and animal, respectively, pooled from serial sections across the full rostral-caudal extent of the ARC, corresponding to planes 26 to 32 as defined by Paxinos and Watson [44] (Figure 1). In order to ensure neuronal specificity, only neurons with a distinct nucleolus and soma appearance were LMD-prepared and used for subsequent measurements [40]. LMD-captured neuronal cells were additionally verified by microscopical inspection of the tube cap.

RNA preparation and quantitative real-time PCR. Total RNA was isolated from LMD-prepared samples and DNase treated using the PureLink RNA Micro Kit (Invitrogen, Carlsbad, USA), according to the manufacturer's protocol as described previously [40]. RNA was reverse-transcribed into complementary DNA (cDNA) using the Superscript-First-Strand-Synthesis System (Invitrogen), and cDNA was amplified in subsequent real-time PCR.

Duplex real-time PCR was performed in triplicate in an Applied Biosystems 7500 instrument [35,40]. *Npy*, *Gal*, *Agrp*, and *Pomc* mRNA expression were analyzed using commercial intron-spanning TaqMan® gene expression assays from Applied Biosystems (*Npy*: Rn00561681_m1; *Gal*: Rn01501525_m1; *Pomc*: Rn00595020_m1; *Agrp*: Rn01431703_g1; all FAM-labeled), together with an endogenous control assay for the housekeeping gene *Beta actin* (4352340E, VIC-labeled), validated previously [40]. For all amplifications, a standard protocol was used: 1 cycle of 95°C for 10 min, followed by 40 two-step cycles at 95°C for 15 s and 60°C for 1 min [TaqMan Gene Expression Assays Protocol, part number 4333458, Applied Biosystems]. Relative expression of target genes (*vs. Beta actin*) was determined by respective C_t values according to the $2^{-\Delta Ct}$ method as described elsewhere [40,45,46].

Statistics

Data are expressed as means ± SD unless otherwise indicated. Real-time PCR data are given as arbitrary units. One-way analysis of variance (one-way ANOVA over all groups) followed by Tukey's HSD *post hoc* analysis was used to analyze group differences (SPSS Software 19.0, Munich, Germany). For analysis of relations between two variables, Spearman's rank correlation test was performed with GraphPad Prism Version 4.03 (GraphPad Software, Inc., San Diego, California, USA). Statistical significance was set at $p<0.05$.

Results

Mortality

Mortality over the entire study period (day 1–560 of life) was increased in neonatally overfed SGA-in-SL rats (12.5%; 2 of 16) as compared to the normal-fed AGA-in-NL rats of the control group (8.5%; 6 of 71), while mortality in neonatally normal-fed SGA-in-NL rats was rather decreased (0%; 0 of 14; differences not statistically significant).

Body Weight and Body Composition

Mean birth weight of rats born small-for-gestational-age (SGA) was significantly decreased as compared to control rats that had a birth weight appropriate-for-gestational-age (AGA) ($p<0.001$; Table 1). SGA rats exposed to neonatal overnutrition by rearing in small litters (SGA-in-SL) showed rapid neonatal weight gain. From day 7 of life onwards, they did not show any further difference in body weight, as compared to AGA pups raised in normal litters. In contrast, SGA pups raised in normal litters (SGA-in-NL) did not catch up in body weight until day 60 of life. After day 60, no further differences in body weight were observed among the three groups. Additionally, no significant differences in body length and relative body weight were observed in SGA rats raised in normal *vs.* small litters as compared to the rats of the control group until the end of the study (Table 1).

Analysis of body composition revealed no significant group differences in fat-free dry-mass at day 560, *i.e.*, at the end of the study. However, a trend towards increased body fat content was observed in neonatally overfed SGA-in-SL rats (Table 1).

Basal Metabolic Parameters

While basal, *i.e.*, fasting blood glucose levels did not differ significantly between groups over the entire observational period, plasma insulin concentrations were significantly increased in neonatally overnourished SGA-in-SL rats on days 360 of life, *i.e.*, before the feeding study, and 560 of life, *i.e.*, at the end of the HE/HF feeding study, as compared to AGA-in-NL control rats (day 360: AGA-in-NL: 29.3±10.6 mIU/l *vs.* SGA-in-NL:

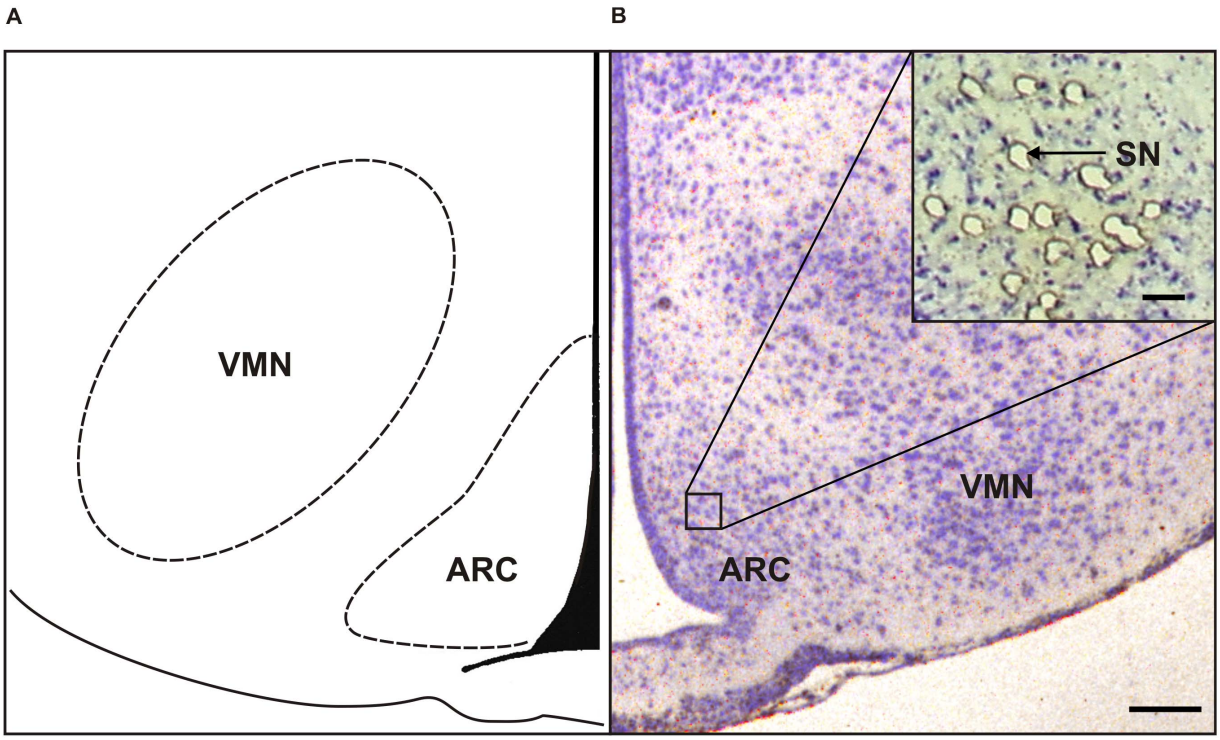


Figure 1. Single neuron preparation using lasercapture microdissection. Schematic illustration (**A**) and Nissl-staining (**B**) of rat hypothalamic nuclei at plane 29 according to Paxinos and Watson [44]; scale bar = 100 µm. Insert shows single neuron preparations from the arcuate nucleus (ARC) using Lasercapture microdissection (LMD); scale bar = 25 µm. VMN = ventromedial hypothalamic nucleus, SN = microdissected single neuron.
doi:10.1371/journal.pone.0078799.g001

30.5±9.7 mIU/l *vs.* SGA-in-SL: 39.9±13.7 mIU/l; day 560: AGA-in-NL: 84.8±34.2 mIU/l *vs.* SGA-in-NL: 82.7±30.4 mIU/l *vs.* SGA-in-SL: 113.7±62.3 mIU/l; both $p<0.05$; Figure 2A and 2B). Basal hyperinsulinemia was associated with a significantly increased insulin/glucose-ratio in SGA-in-SL rats (day 360: AGA-in-NL: 6.4±2.3 *vs.* SGA-in-NL: 7.6±2.6 *vs.* SGA-in-SL: 8.5±2.8; day 560: AGA-in-NL: 15.4±6.3 *vs.* SGA-in-NL: 14.6±5.3 *vs.* SGA-in-SL: 21.6±11.3; both $p<0.05$; Figure 2A and 2B). This was accompanied by hyperleptinemia ($p<0.01$), significantly correlated with body fat (Figure 3A), significantly increased cholesterol levels ($p<0.05$) and slightly increased levels of triglycerides at day 560 of life in SGA-in-SL rats. In contrast, at no time point were significant differences in metabolic profile found in SGA rats raised in normal litters (SGA-in-NL) as compared to controls (AGA-in-NL).

Glucose Tolerance Test

After intraperitoneal glucose loading at younger adult age (day 130 of life), neonatally overnourished SGA-in-SL rats showed significantly increased blood glucose levels at 90 minutes ($p<0.05$; Figure 2C), while their AUCG was not significantly increased (AGA-in-NL: 15.8±2.7 mmol/l/h *vs.* SGA-in-NL: 16.8±2.8 mmol/l/h *vs.* SGA-in-SL: 17.2±2.4 mmol/l/h; $p = 0.097$). Glucose tolerance test at later adult age (day 530 of life) revealed no significant differences between groups (Figure 2D).

Food Intake

Energy intake in adulthood of standard laboratory chow (days 470–500 of life) was significantly decreased in neonatally normal-fed SGA rats (SGA-in-NL) as compared to both SGA-in-SL and AGA-in-NL rats (AGA-in-NL: 57.9±9.0 kcal/d *vs.* SGA-in-NL: 50.1±6.1 kcal/d *vs.* SGA-in-SL: 59.4±5.3 kcal/d; $p<0.05$ and $p<0.01$, respectively; Figure 4A). However, SGA-in-NL rats consumed similar calories of high-energy/high-fat diet (days 500–560 of life) as compared to AGA-in-NL animals, while neonatally overfed SGA rats showed significantly increased energy intake of HE/HF diet (AGA-in-NL: 65.9±6.8 kcal/d *vs.* SGA-in-NL: 65.9±6.2 kcal/d *vs.* SGA-in-SL: 72.0±7.5 kcal/d; $p<0.01$; Figure 4B). Energy intake over the whole observational period (day 470–560 of life) was significantly increased in SGA-in-SL rats as compared to SGA-in-NL rats (AGA-in-NL: 63.5±6.7 kcal/d *vs.* SGA-in-NL: 61.1±5.1 kcal/d *vs.* SGA-in-SL: 68.1±5.9 kcal/d; $p<0.05$; Figure 4C), and positively correlated to body fat over all groups (Figure 3B). Accordingly, leptin was positively correlated to body fat not just in SGA-in-SL ($r = 0.42$) but in all groups (AGA-in-NL: $r = 0.58$; SGA-in-NL: $r = 0.68$; Figure 3A).

Neuropeptide Expression in the Hypothalamic Arcuate Nucleus (ARC)

Analyses of mRNA expression of neuropeptides in single neuron pools (n = 100 neurons *per* animal) from the ARC at the end of the experiment on day 560 of life revealed no significant differences between groups (Figure 5A). Increased overall food intake in neonatally overnourished SGA-in-SL rats, however, was accompanied by a non-significant tendency towards decreased levels of anorexigenic *Pomc* and increased levels of orexigenic *Npy* expression as compared to AGA-in-NL control rats (*Pomc*: AGA-in-NL: 0.86±0.15 *vs.* SGA-in-NL: 1.00±0.17 *vs.* SGA-in-SL: 0.63±0.05, $p = 0.213$; *Npy*: AGA-in-NL: 0.9±0.2 *vs.* SGA-in-NL: 0.7±0.2 *vs.* SGA-in-SL: 1.2±0.1, $p = 0.206$; Figure 5A). Expression of *Agrp* and *Gal* was unchanged. Neonatally normal-fed SGA-in-NL rats exhibited decreased expression (not statistically

Table 1. Body weight, body length and body composition of rats born appropriate-for-gestational-age (AGA) or small-for-gestational-age (SGA).

Variables	AGA-in-NL	SGA-in-NL	SGA-in-SL	*p* (one-way ANOVA)
Body weight (g)				
day 1	6.6±0.5 (71)	5.8±0.5[a] (14)	5.9±0.5[a] (16)	**<0.001**
day 7	14.1±1.4 (71)	12.4±1.6[a] (14)	13.8±2.8 (16)	**0.004**
day 14	28.2±2.2 (71)	25.2±4.2[a] (14)	29.7±6.6[b] (16)	**0.003**
day 21	46.8±4.3 (71)	40.7±5.8[a] (14)	47.3±10.0[b] (16)	**0.001**
day 30	88.7±6.4 (71)	81.6±10.5[a] (14)	86.2±18.3 (16)	**0.044**
day 60	208±23.4 (71)	208±19.9 (14)	206±33.9 (16)	0.951
day 90	260±22.3 (71)	259±22.0 (14)	255±40.3 (16)	0.763
day 180	310±28.1 (71)	308±29.0 (14)	300±50.5 (16)	0.505
day 360	361±33.1 (70)	363±53.8 (14)	362±65.2 (14)	0.990
day 540	432±56.7 (65)	457±62.3 (14)	460±89.1 (14)	0.177
day 560	444±54.9 (65)	461±56.0 (14)	450±58.6 (14)	0.563
Body length (cm)				
day 21	10.1±0.3 (71)	9.9±0.5 (14)	10.3±1.1 (16)	0.108
day 30	13.3±0.4 (71)	12.9±0.6 (14)	13.1±1.3 (16)	0.066
day 60	18.7±0.5 (71)	18.5±0.6 (14)	18.4±1.0 (16)	0.261
day 90	20.2±0.5 (71)	20.1±0.6 (14)	19.9±0.9 (16)	0.170
day 180	21.7±0.6 (71)	21.5±0.8 (14)	21.3±0.7 (16)	0.058
day 360	22.3±0.6 (70)	22.4±0.6 (14)	22.2±0.9 (14)	0.587
day 540	22.2±0.5 (65)	22.2±0.5 (14)	21.9±0.7 (14)	0.117
day 560	22.2±0.5 (65)	22.3±0.5 (14)	21.9±0.7 (14)	0.092
Body composition (day 560)				
Relative body weight (g/cm)	20.0±2.2 (65)	20.4±2.0 (14)	20.6±2.3 (14)	0.543
Fat-free dry-mass (%)	19.4±2.0 (64)	19.3±1.8 (14)	19.0±2.6 (14)	0.809
Body fat content (%)	32.5±6.0 (64)	32.0±5.8 (14)	35.5±7.2 (14)	0.223

Values are expressed as means ± SD. Number of animals in parenthesis.
[a]$p<0.05$ *vs*. AGA-in-NL,
[b]$p<0.05$ *vs*. SGA-in-NL (by Tukey's HSD *post hoc* analyses).
Abbreviation: NL, normal litter; SL, small litter.
doi:10.1371/journal.pone.0078799.t001

significant) of orexigenic *Npy*, *Agrp* and *Gal* (*Agrp*: AGA-in-NL: 0.15±0.03 *vs*. SGA-in-NL: 0.11±0.03 *vs*. SGA-in-SL: 0.14±0.01, $p = 0.525$; *Gal*: AGA-in-NL: 0.08±0.01 *vs*. SGA-in-NL: 0.05±0.01 *vs*. SGA-in-SL: 0.08±0.02, $p = 0.306$; Figure 5A), corresponding to their overall tendency towards reduced food intake under chow diet (Figure 4).

Because of the well-known dependency of neuropeptide expression (*Pomc*, *Agrp*, *Npy*, *Gal*) on their regulating hormones leptin and insulin [47–50], we additionally calculated the quotient of neuropeptide expression *per* unit of leptin and insulin, respectively, as described elsewhere [32]. In SGA-in-SL rats, *Pomc* expression *per* corresponding insulin was clearly decreased as compared to AGA-in-NL rats (AGA-in-NL: 13.6±1.9 *vs*. SGA-in-NL: 12.2±2.6 *vs*. SGA-in-SL: 6.0±0.4; $p<0.05$), whereas the above mentioned non-significant increase in *Npy* expression was no longer present (AGA-in-NL: 13.8±1.8 *vs*. SGA-in-NL: 9.2±1.9 *vs*. SGA-in-SL: 13.5±1.5; $p = 0.158$; Figure 5B). In contrast, SGA-in-NL rats showed a marked decrease in *Agrp* expression *per* corresponding leptin as compared to controls (AGA-in-NL: 10.9±2.4 *vs*. SGA-in-NL: 4.8±1.0 *vs*. SGA-in-SL: 6.1±0.8; $p<0.05$; Figure 5C). In general, the trend towards decreased expression of orexigenic *Npy*, *Agrp*, and *Gal* in SGA-in-NL rats remained even when referring to insulin and leptin, respectively (Figure 5B and 5C). Expression of anorexigenic *Pomc* was unchanged in SGA-in-NL rats, even when referred to insulin and leptin, respectively (Figure 5B and 5C).

Finally, quotients of orexigenic (*Agrp*, *Npy*, *Gal*) *per* anorexigenic (*Pomc*) mRNA expression were calculated to get a proxy of the 'net-balance' here [Figure 5D]. *Gal*/*Pomc* was increased significantly in SGA-in-SL as compared to SGA-in-NL rats. Moreover, *Npy*/*Pomc* was found to be nearly doubled in SGA-in-SL as compared to both AGA controls as well as SGA-in-NL rats, and found to be positively correlated to food intake over all groups (Figure 3C).

Discussion

Low birth weight (IUGR, SGA) has been shown to be related to increased long-term 'diabesity' risk though reasons remain unclear. Acquired alterations of food intake have been suggested as a possible mechanism. In most of epidemiological-clinical studies, *in utero* causes of low birth weight and, especially, the potential impact of neonatal nutrition and resulting early growth pattern for the long-term outcome have not been adequately considered. We investigated the impact of neonatal overfeeding for the long-term 'diabesity' risk, food intake, and related neuropeptidergic regulatory parameters of body weight control in 'small-for-

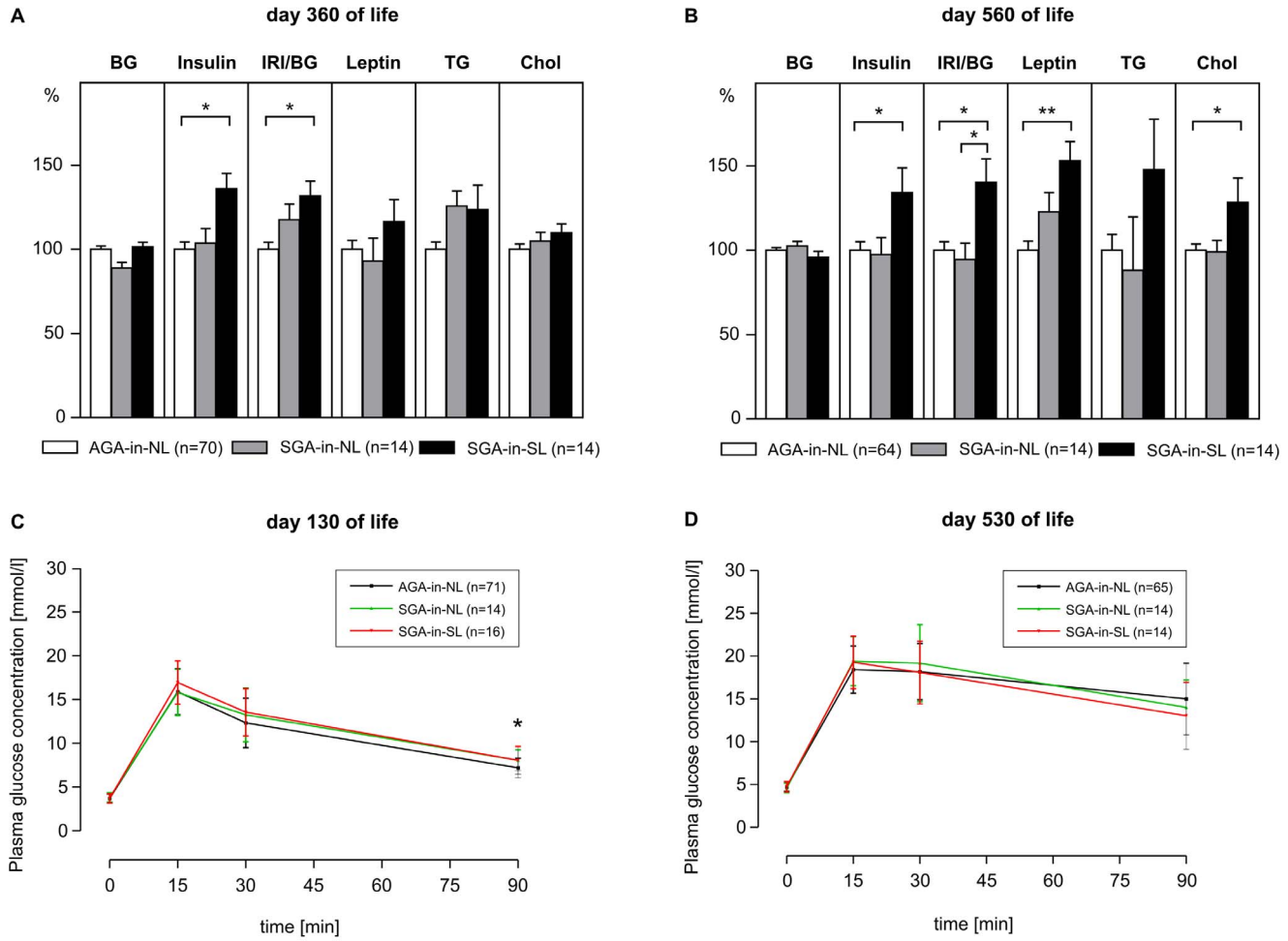


Figure 2. Metabolic parameters in early and later adulthood. Fasting plasma levels of blood glucose (BG), insulin, insulin/glucose-ratio (IRI/BG), leptin, triglycerides (TG), and cholesterol (Chol) on day 360 of life, *i.e.*, before the feeding study (**A**), and day 560 of life, *i.e.*, at the end of HE/HF feeding study (**B**). Data are shown as percentages of AGA-in-NL-levels (means ± SEM). Plasma glucose levels after intraperitoneal glucose loading on day 130 of life, *i.e.*, before feeding study (**C**), and day 530 of life, *i.e.*, during high-energy/high-fat (HE/HF) feeding study (**D**) in rats born small-for-gestational-age, raised in normal litters (SGA-in-NL) or small litters (SGA-in-SL), as compared to rats with normal birth weight raised in normal litters (AGA-in-NL). Data are means ± SD. *$p<0.05$, **$p<0.01$ (one-way ANOVA followed by Tukey's HSD *post hoc* analysis).
doi:10.1371/journal.pone.0078799.g002

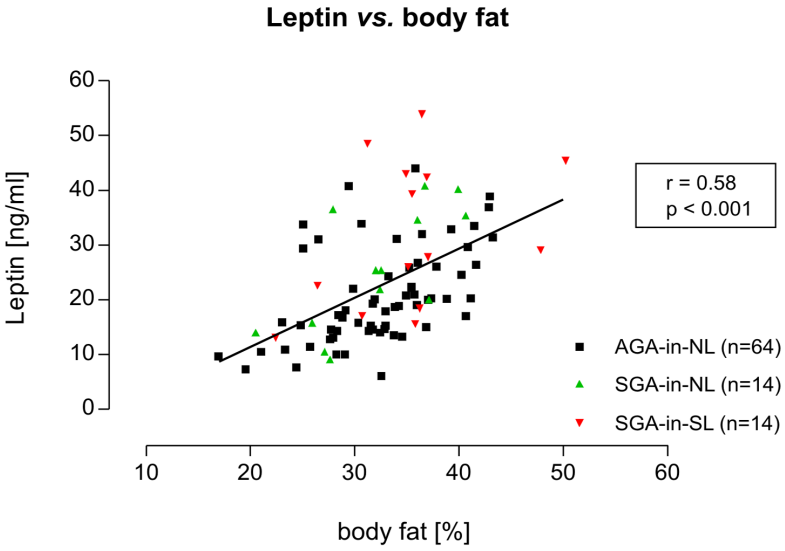
gestational-age' rats, introducing a novel 'genuine' rodent LBW-model set according to clinical definitions of SGA (birth weight below the lower limit of the 95% confidence interval of the mean birth weight of same litter and sex).

In our study, all animals with a reduced birth weight (SGA) showed catch-up growth, irrespective of whether they were neonatally normal-fed or overfed. Adult age SGA rats did not differ in total body weight and body length when compared to AGA control rats. This is consistent with epidemiological observations which described that 80–90% of SGA newborns show full catch-up growth within the first two years of life [51,52]. However, while neonatally normal-fed SGA pups only caught up in body weight at day 60 of life, neonatal overnutrition of SGA pups resulted in 'rapid' neonatal weight gain within some days. From the first week of life onwards, these SGA-in-SL rats did not further differ from AGA controls.

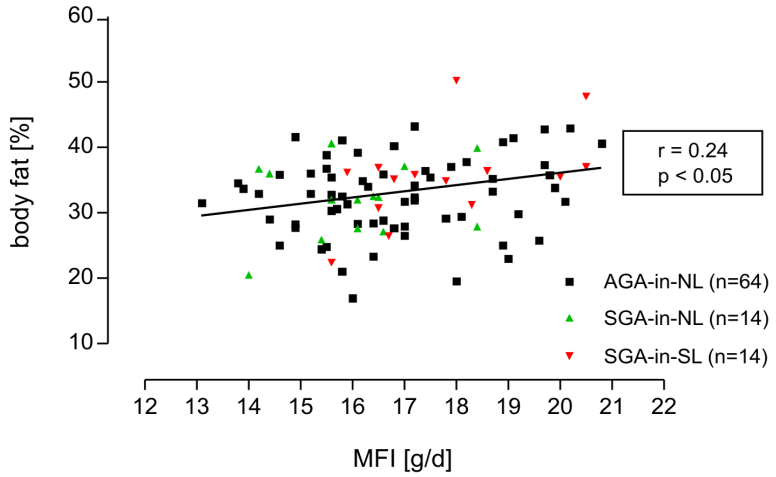
Epidemiological-clinical studies have indicated that 'rapid' neonatal weight gain appears to be a risk factor for the development of obesity, increased body fat, insulin resistance, impaired glucose tolerance and cardiovascular diseases in the long-term [8,20,53–58], even independent of birth weight [23,59]. In a large population-based study, Stettler and colleagues examined the extent to which the development of overweight depends on birth weight and/or weight gain during the first 4 months of life. They observed that increased weight gain from birth until the age of 4 months is associated with later increased overweight risk [59]. This association has been completely confirmed in prenatally 'overfed' (hyperglycemia-exposed) offspring of diabetic mothers [23]. By these studies, rapid neonatal weight gain has been shown to be an *independent* risk factor in general as well as in particular at risk populations [23,59].

According to the 'small-baby-syndrome' hypothesis [6], children with a low birth weight are *per se* at increased risk to develop metabolic disturbances in later life, *e.g.*, impaired glucose tolerance and dyslipidemia, resulting from prenatal undernutrition. Neonatally normal-fed SGA rats in the present study, however, did not show increased risk throughout later life. In contrast, during glucose tolerance test at day 130 of life, neonatally overnourished SGA-in-SL rats showed significantly increased blood glucose levels at 90 minutes. This tendency towards altered metabolism became further accentuated in older animals, when neonatally overfed SGA-in-SL rats developed hyperinsulinemia and an increased

A


Leptin *vs.* body fat
Leptin [ng/ml]
body fat [%]
r = 0.58
p < 0.001
AGA-in-NL (n=64)
SGA-in-NL (n=14)
SGA-in-SL (n=14)

B


body fat *vs.* mean food intake
body fat [%]
MFI [g/d]
r = 0.24
p < 0.05
AGA-in-NL (n=64)
SGA-in-NL (n=14)
SGA-in-SL (n=14)

C

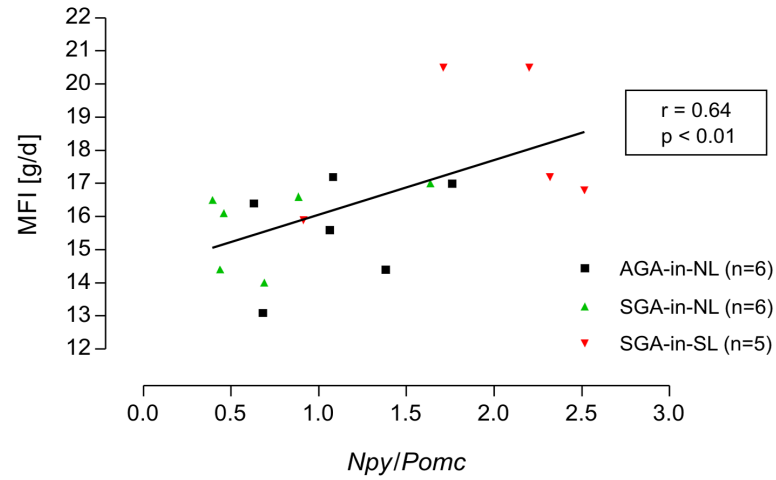

mean food intake *vs. Npy/Pomc* mRNA
MFI [g/d]
Npy/Pomc
r = 0.64
p < 0.01
AGA-in-NL (n=6)
SGA-in-NL (n=6)
SGA-in-SL (n=5)

Figure 3. Correlation analyses. Relation between plasma levels of leptin and percentage of body fat at day 560 of life (**A**). Percentage of body fat presented as a function of overall mean food intake (chow+HE/HF) (**B**). Relation between overall mean food intake (chow+HE/HF diet) and mRNA expression of *Npy per* unit *Pomc* (*Npy*/*Pomc*) in the arcuate hypothalamic nucleus at day 560 of life (**C**). Group-specific plots are illustrated (■: AGA-in-NL; ▲: SGA-in-NL; ▼: SGA-in-SL). Inserts show overall correlation coefficients and significances derived from Spearman's rank correlation tests.
doi:10.1371/journal.pone.0078799.g003

insulin/glucose ratio under basal conditions (days 360 and 560 of life), indicating insulin resistance [42]. Consequently, metabolic alterations observed in SGA rats cannot be attributed to decreased birth weight *per se*, but suggest rather early postnatal overnutrition as the critical risk factor here. This appears to be in line with findings of a clinical study in which the influence of prematurity on later occurrence of insulin resistance has been studied [9]. Hofman et al. observed that children born with low birth weight had reduced insulin sensitivity later on, irrespective of their gestational age (preterm AGA or term SGA). They found that the risk among preterm AGA-children was similar to the risk of term SGA-children. This observation gives rise to reasonable doubts concerning the role of diminished prenatal food supply as an *independent* risk factor in the pathogenesis of 'diabesity' in the 'small-baby-syndrome' [60]. It can be assumed that rather postnatal influences, especially neonatal overnutrition leading to rapid neonatal weight gain, are of critical importance for the long-term 'diabesogenic' outcome of 'SGA babies'. Accordingly, the association between rapid neonatal weight gain and later metabolic disorders was examined in a prospective cohort study. Fabricius-Bjerre et al. observed that accelerated growth during the first three months of life leads to disturbances of glucose metabolism later in life [8]. High infant weight gain was positively related with high insulin levels as well as high HOMA-IR later on (homeostasis model assessment of insulin resistance).

In addition to the above mentioned diabetic alterations at older adult age in neonatally overfed SGA rats, these animals also showed an altered fat metabolism. SGA-in-SL rats displayed increased levels of cholesterol and triglycerides and clearly increased leptin levels, while SGA-in-NL rats did not show any adipogenic alterations. Hyperleptinemia in neonatally overnourished rats was accompanied by a tendency towards increased body fat content which was, however, not associated with significantly increased total body weight, finally indicating increased 'adiposity' in these animals. Thus, elevated leptin levels in SGA-in-SL appear to reflect increased body fat, not necessarily increased total body weight, which is underlined by a positive correlation of body fat with plasma leptin levels (Figure 3A). Findings from human studies support this relationship. For instance, Ibánez et al. have demonstrated that children who were born SGA at term have abdominal fat mass at 4 years closely related to the rate of catch-up weight gain within the first 2 years of life [57]. It is well known from a number of clinical studies that increased fat mass decisively contributes to the development of insulin resistance [61–63], accompanied with increased plasma insulin and triglyceride levels [63], as observed here experimentally.

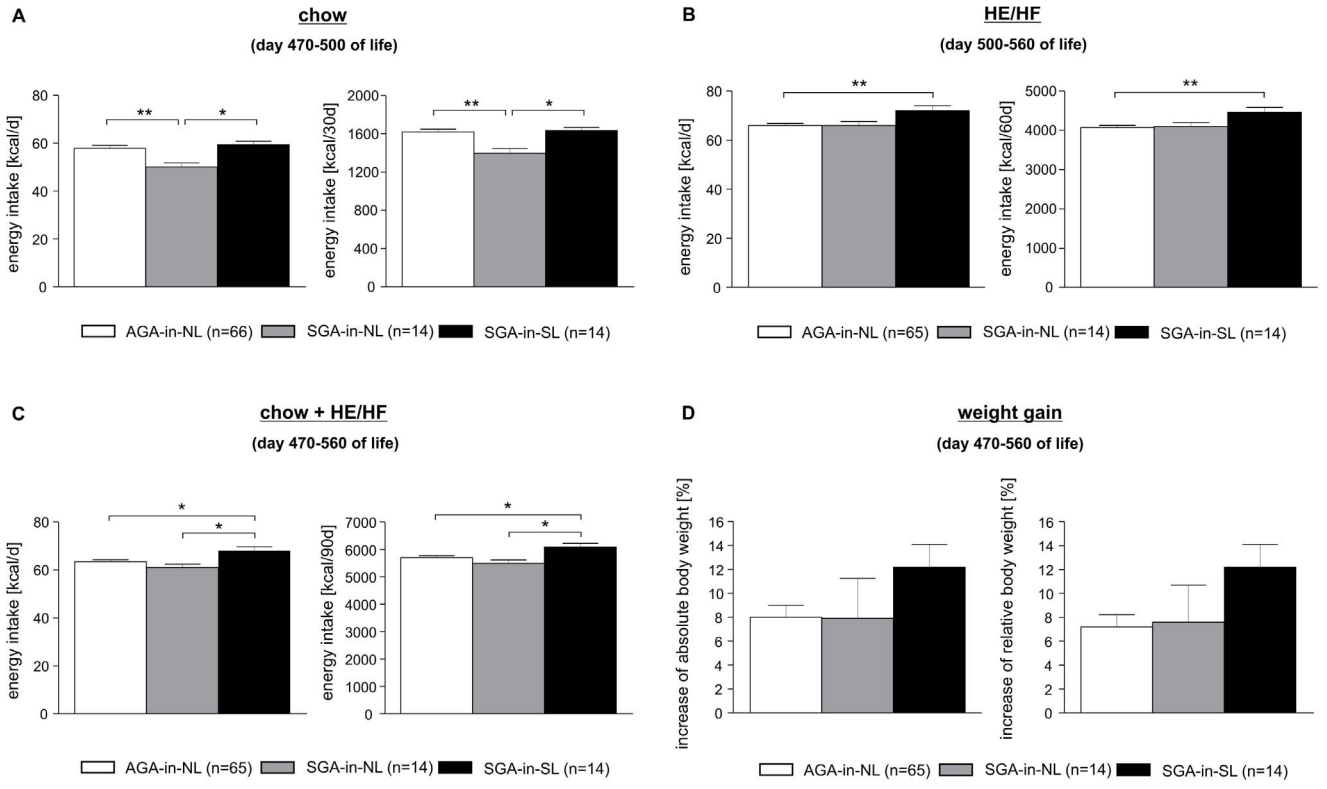


Figure 4. Food intake study in later adult age. *Ad libitum* energy intake of standard laboratory chow for 30 days (day 470–500 of life) (**A**), followed by providing a high-energy/high-fat (HE/HF) diet for 60 days (day 500–560 of life) (**B**). (**C**) shows overall caloric intake of chow and HE/HF diet throughout food intake study (day 470–560 of life). Absolute and relative body weight changes during the food intake study (day 470–560 of life) (**D**). Data are means ± SEM, shown as percentages of AGA-in-NL-levels. *$p<0.05$, **$p<0.01$ (one-way ANOVA followed by Tukey's HSD *post hoc* analysis).
doi:10.1371/journal.pone.0078799.g004

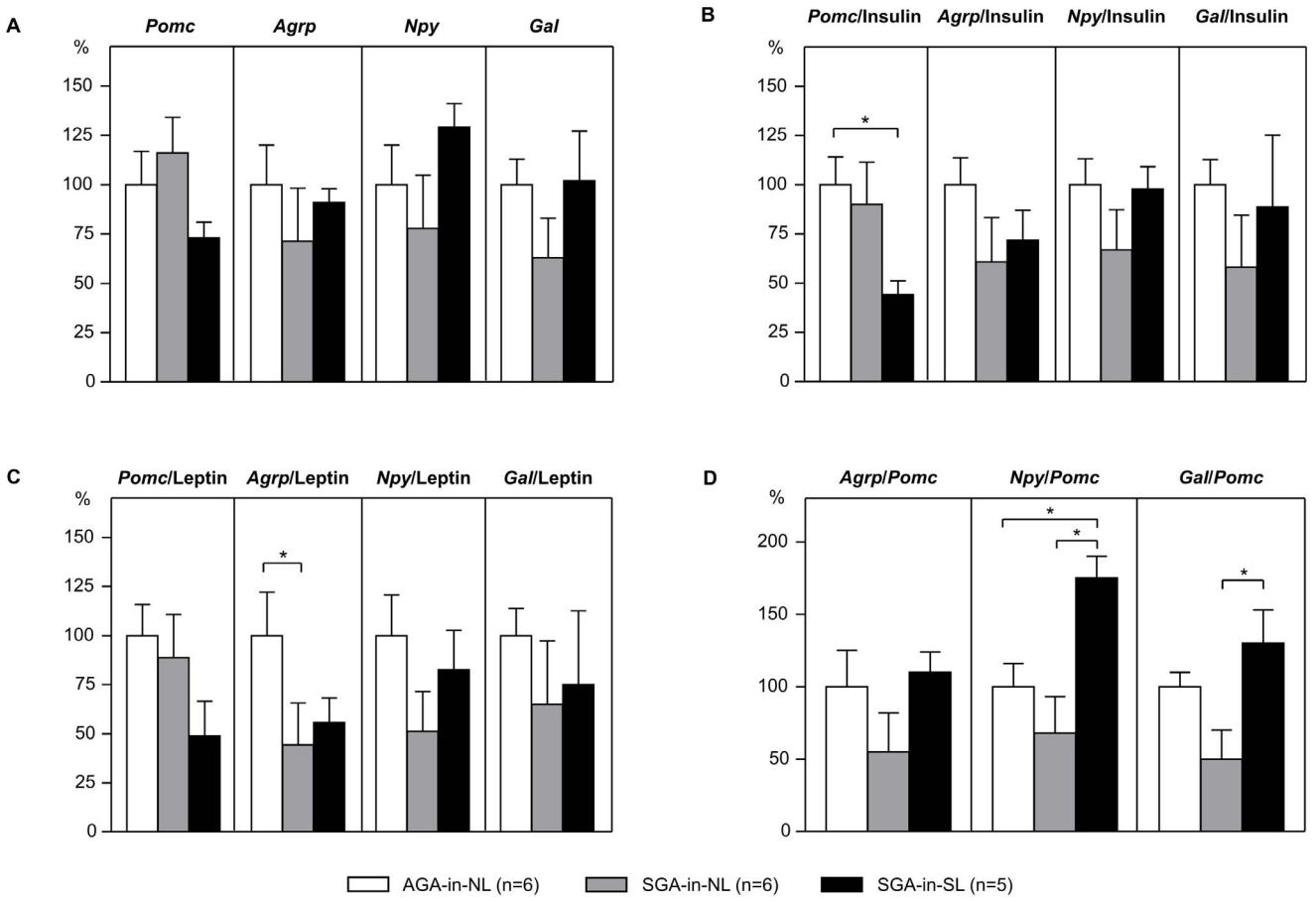


Figure 5. Neuropeptide mRNA expression in neuron pools of the hypothalamic ARC nucleus at day 560 of life. Relative gene expression of *proopiomelanocortin* (*Pomc*), *agouti-related peptide* (*Agrp*), *neuropeptide Y* (*Npy*), and *galanin* (*Gal*), all normalized to *Beta actin* (**A**). Additionally, expression levels at the end of the feeding study (day 560 of life) are shown *per* unit plasma insulin (**B**) and plasma leptin (**C**), respectively, in AGA and SGA rats raised in normal (NL) or small litters (SL). Illustration of 'orexigenic indices', calculated as quotients of orexigenic (*Agrp*, *Npy*, *Gal*) *per* anorexigenic (*Pomc*) mRNA expression (**D**). Data are means ± SEM, shown as percentages of AGA-in-NL-levels. *$p<0.05$ (one-way ANOVA followed by Tukey's HSD *post hoc* analysis).
doi:10.1371/journal.pone.0078799.g005

In the context of the pathogenesis of the 'small-baby-syndrome', a recently published cohort study considered altered dietary habits in later life to be causal for adverse health outcomes. Being small at birth was associated with higher intake of fat at later adult age [11]. This confirms findings of another longitudinal study in which an inverse relation between birth weight and fat intake in 43-month-old children was described [64]. Interestingly, these epidemiological findings seem to be confirmed by data from our animal experiment. However, the clinical studies did not consider neonatal nutrition and growth pattern. In our experimental study, only SGA rats which were neonatally overfed showed increased food intake later on, especially under high-energy/high-fat diet. Worthy to note, milk of SL dams has been characterized to be altered in terms of a high-energy/high-fat quality as compared to milk of dams nourishing litters of normal size [29]. Therefore, our observations might even indicate an early food preference conditioning of SGA-in-SL rats through their dams' milk composition towards a HE/HF diet preference, persisting throughout later life. Trend towards elevated body fat content observed in later life of SGA-in-SL rats was possibly caused by hyperphagia and high-energy/high-fat food preference, confirmed by positive correlation between body fat and food intake (Figure 3B). In contrast, neonatally normal-fed SGA-in-NL rats did not show hyperphagia, neither under chow nor under high-energy/high-fat diet. However, a trend towards 'relative preference' for HE/HF diet *vs.* chow was observed within the SGA-in-NL group, although neither indicating hyperphagia nor HE/HF preference as compared to AGA or SGA-in-SL rats (Figure 4).

In the presented study, neonatally normal- and overfed SGA rats did not differ significantly with respect to mortality. However, in SGA-in-NL rats mortality was trending to be even lower as compared to AGA controls while, in contrast, SGA-in-SL rats showed a tendency towards increased mortality. This appears to be in line with findings by Ozanne and Hales [65]. While in their studies pre- and neonatal underfeeding gave rise to increased longevity, decreased life span of male mice that underwent fetal growth restriction and thereafter experienced rapid catch-up growth has been observed [65]. Note, in the mentioned experiment [65] reduced birth weight was induced by maternal protein-restriction during pregnancy. Rodent models of maternal malnutrition during pregnancy and lactation are among the most frequently used animal models to investigate mechanisms of perinatal programming according to the 'small-baby-syndrome' hypothesis. However, offspring whose dams were fed a low protein diet during pregnancy did not always exhibit the full spectrum of metabolic and cardiovascular alterations as observed in epidemi-

ological and clinical studies. Moreover, experiments mostly were carried out exclusively in males and/or offspring were not examined into older adult age [18,19,65]. Therefore, in our study we focused on females and later adult aged animals to properly examine the long-term outcomes.

Food intake and body weight are decisively regulated by orexigenic and anorexigenic neuropeptides expressed in the hypothalamic arcuate nucleus (ARC) [66]. The hypothalamic expression of these neuropeptides is mainly regulated by the circulating satiety signals leptin and insulin [50]. Lasercapture microdissection of single neurons combined with quantitative PCR has been proven to be the most powerful technique allowing a highly precise and complex analysis of gene expression patterns in discrete neuronal cell populations [40]. Therefore, we applied this method here to perform gene expression analyses. Measurements at older adult age revealed a trend towards down-regulation of orexigenic *Agrp* and *Npy* in neonatally normal-fed SGA-in-NL rats whereas expression of the anorexigenic *Pomc* was slightly increased. Expression of orexigenic *Gal* was rather decreased in SGA-in-NL. This is of particular interest since *Gal* is known to particularly stimulate fat ingestion [48,67]. Altogether, results show a long-term *decreased* activity of the orexigenic system in neonatally normal-fed SGA-in-NL rats, which is strengthened by consideration of circulating leptin and insulin levels (Figures 5B and 5C) and might be causal for the significantly decreased food intake as compared to controls, especially under chow diet.

In contrast, hypothalamic expression of *Agrp* and *Gal* was unchanged and *Npy* even slightly increased in neonatally overfed SGA-in-SL rats as compared to AGA controls, despite their marked basal hyperleptinemia and hyperinsulinemia. Corresponding expression of the anorexigenic *Pomc* was even decreased, also when referred to the increased levels of the satiety signals insulin and leptin. This gene expression pattern strongly indicates a neonatally acquired neuropeptidergic malprogramming, especially of the anorexigenic *Pomc*-system, due to neonatal overfeeding in SGA rats (SGA-in-SL).

Finally, since food intake is regulated by both orexigenic and anorexigenic neuropeptides, we additionally introduced here an integrative neuropeptidergic 'net-indicator'. Dividing the expression levels of *Npy*, the most potent orexigenic neuropeptide [66], by the expression levels of *Pomc*, the most important anorexigenic neuropeptide [66], may provide an orientating proxy ('orexigenic index') for better estimation of neuropeptidergic appetite *vs.* satiety activity and regulation in a given situation. In neonatally overfed SGA-in-SL rats, reduced expression of anorexigenic *Pomc* and unchanged expression of orexigenic *Npy* resulted in an increased 'orexigenic index' (*Npy*/*Pomc*), corresponding with hyperphagia and supported by a positive correlation with overall food intake. Similar was observed for the *Gal*/*Pomc* index (Figure 5D). In contrast, in neonatally normal-fed SGA-in-NL no respective alterations were observed as compared to AGA controls (Figure 5D).

In summary, neonatally normal-fed SGA rats (SGA-in-NL) growth caught up only at late juvenile age and did not develop 'diabesity' and hyperphagia later on, neither under normal chow diet nor under high-energy/high-fat dietary provocation representing a 'westernized' lifestyle. Their long-term hypothalamically driven orexigenic activity was rather decreased than increased. In contrast, neonatally overfed SGA-in-SL rats displayed rapid neonatal weight gain and catch-up growth within the first week of postnatal life. In the long-term, these SGA rats displayed significantly increased 'diabesity' risk as compared to normal rats. Hyperphagia, particularly pronounced under high-energy/high-fat dietary provocation, was accompanied with hyperleptinemia, hyperinsulinemia, increased insulin-glucose-ratio, and correlated with body fat. This was accompanied with and correlated to reduced expression of the anorexigenic hypothalamic ARC-*Pomc*, and respective increase of the 'orexigenic index' (*Npy*/*Pomc*, *Gal*/*Pomc*), even under consideration of the circulating regulators insulin and leptin. Altogether, this indicates a neonatally acquired hypothalamic resistance of the anorexigenic system towards peripheral satiety signals (insulin, leptin) in neonatally overfed SGA-in-SL rats.

In conclusion, the early neonatal period appears to be at least as critical as prenatal life for long-term programming of 'diabesity' risk and altered food intake in SGA rats, as we previously suggested and proposed [16,20,31,33,39]. Neonatal overfeeding may predispose *via* hypothalamic malprogramming to hyperphagia and accompanying/subsequent disorders in terms of the metabolic syndrome in 'small-for-gestational-age' subjects. This should be considered in future experimental as well as clinical approaches to unravel mechanisms underlying the 'small-baby-syndrome'.

Acknowledgments

We would like to thank Kerstin Melchior and Thomas Ziska for excellent technical assistance, and Thomas Harder, MD, MScE, for help with statistics.

Author Contributions

Conceived and designed the experiments: AP. Performed the experiments: KS UN RR. Analyzed the data: KS UN RR AP. Contributed reagents/materials/analysis tools: KS UN RR AP. Wrote the paper: KS UN RR AP.

References

1. Flegal KM, Carroll MD, Kit BK, Ogden CL (2012) Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999–2010. JAMA 307: 491–497.
2. Hossain P, Kawar B, El Nahas M (2007) Obesity and diabetes in the developing world – a growing challenge. N Engl J Med 356: 213–215.
3. Ogden CL, Carroll MD, Kit BK, Flegal KM (2012) Prevalence of obesity and trends in body mass index among US children and adolescents, 1999–2010. JAMA 307: 483–490.
4. Scully T (2012) Diabetes in numbers. Nature 485: S2–3.
5. Hales CN, Barker DJP (1992) Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia 35: 595–601.
6. Barker DJP, Hales CN, Fall CHD, Osmond C, Phipps K, et al. (1993) Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia 36: 62–67.
7. Hales CN, Barker DJP (2001) The thrifty phenotype hypothesis. Br Med Bull 60: 5–20.
8. Fabricius-Bjerre S, Jensen RB, Færch K, Larsen T, Mølgaard C, et al. (2011) Impact of birth weight and early infant weight gain on insulin resistance and associated cardiovascular risk factors in adolescence. PLoS One 6: e20595.
9. Hofman PL, Regan F, Jackson WE, Jefferies C, Knight DB, et al. (2004) Premature birth and later insulin resistance. N Engl J Med 351: 2179–2186.
10. Levy-Marchal C, Jaquet D (2004) Long-term metabolic consequences of being born for gestational age. Pediatr Diabetes 5: 147–153.
11. Perälä MM, Männistö S, Kaartinen NE, Kajantie E, Osmond C, et al. (2012) Body size at birth is associated with food and nutrient intake in adulthood. PLoS One 7: e46139.
12. Harder T, Rodekamp E, Schellong K, Dudenhausen JW, Plagemann A (2007) Birth weight and subsequent risk of type 2 diabetes: a meta-analysis. Am J Epidemiol 165: 849–857.
13. Huxley R, Neil A, Collins R (2002) Unravelling the fetal origins hypothesis: is there really an inverse association between birthweight and subsequent blood pressure? Lancet 360: 659–665.
14. Gamborg M, Byberg L, Rasmussen F, Andersen PK, Baker JL, et al. (2007) Birth weight and systolic blood pressure in adolescence and adulthood: Meta-

regression analysis of sex- and age-specific results from 20 Nordic studies. Am J Epidemiol 166: 634–645.
15. Schellong K, Schulz S, Harder T, Plagemann A (2012) Birth weight and long-term overweight risk: systematic review and a meta-analysis including 643,902 persons from 66 studies and 26 countries globally. PLoS One 7: e47776.
16. Plagemann A, Harder T, Schellong K, Schulz S, Stupin JH (2012) Early postnatal life as a critical time window for determination of long-term metabolic health. Best Pract Res Clin Endocrinol Metab 26: 641–653.
17. Holemans K, Gerber R, Meurrens K, De Clerck F, Poston L, et al. (1999) Maternal food restriction in the second half of pregnancy affects vascular function but not blood pressure of rat female offspring. Br J Nutr 81: 73–79.
18. Ozanne SE, Wang CL, Dorling MW, Petry CJ (1999) Dissection of the metabolic actions of insulin in adipocytes from early growth-retarded male rats. J Endocrinol 162: 313–319.
19. Petry CJ, Ozanne SE, Wang CL, Hales CN (1997) Early protein restriction and obesity independently induce hypertension in 1-year-old rats. Clin Sci 93: 147–152.
20. Dörner G, Plagemann A (1994) Perinatal hyperinsulinism as possible predisposing factor for diabetes mellitus, obesity and enhanced cardiovascular risk in later life. Horm Metab Res 26: 213–221.
21. Crowther NJ, Trusler J, Cameron N, Toman M, Gray IP (2000) Relation between weight gain and beta-cell secretory activity and non-esterified fatty acid production in 7-year old African children: results from the birth to Ten study. Diabetologia 43: 978–985.
22. Fewtrell MS, Doherty C, Cole TJ, Stafford M, Hales CN, et al. (2000) Effects of size at birth, gestational age and early growth in preterm infants on glucose and insulin concentrations at 9–12 years. Diabetologia 43: 714–717.
23. Plagemann A, Harder T, Rodekamp E, Kohlhoff R (2012) Rapid neonatal weight gain increases risk of childhood overweight in offspring of diabetic mothers. J Perinat Med 40: 557–563.
24. Caruso V, Chen H, Morris MJ (2011) Early hypothalamic FTO overexpression in response to maternal obesity – potential contribution to postweaning hyperphagia. PLoS One 6: e25261.
25. Hou M, Liu Y, Zhu L, Sun B, Guo M, et al. (2011) Neonatal overfeeding induced by small litter rearing causes altered glucocorticoid metabolism in rats. PLoS One 6: e25726.
26. López M, Seoane LM, Tovar S, García MC, Nogueiras R, et al. (2005) A possible role of neuropeptide Y, agouti-related protein and leptin receptor isoforms in hypothalamic programming by perinatal feeding in the rat. Diabetologia 48: 140–148.
27. Plagemann A, Harder T, Rake A, Waas T, Melchior K, et al. (1999b) Observations on the orexigenic hypothalamic neuropeptide Y-system in neonatally overfed weanling rats. J Neuroendocrinol 11: 541–546.
28. Rodrigues AL, de Moura EG, Passos MC, Dutra SC, Lisboa PC (2009) Postnatal early overnutrition changes the leptin signalling pathway in the hypothalamic-pituitary-thyroid axis of young and adult rats. J Physiol 587.11: 2647–2661.
29. Fiorotto ML, Burrin DG, Perez M, Reeds PJ (1991) Intake and use of milk nutrients by rat pups suckled in small, medium, or large litters. Am J Physiol 260: R1104–R1113.
30. Boullu-Ciocca S, Dutour A, Guillaume V, Achard V, Oliver C, et al. (2005) Postnatal diet-induced obesity in rats upregulates systemic and adipose tissue glucocorticoid metabolism during development and in adulthood. Diabetes 54: 197–203.
31. Plagemann A, Harder T, Rake A, Voits M, Fink H, et al. (1999a) Perinatal elevation of hypothalamic insulin, acquired malformation of hypothalamic galaninergic neurons, and syndrom X-like alterations in adulthood of neonatally overfed rats. Brain Res 836: 146–155.
32. Plagemann A, Harder T, Brunn M, Harder A, Roepke K, et al. (2009) Hypothalamic proopiomelanocortin promoter methylation becomes altered by early overfeeding: an epigenetic model of obesity and the metabolic syndrome. J Physiol 587.20: 4963–4976.
33. Plagemann A (2005) Perinatal programming and functional teratogenesis: impact on body weight regulation and obesity. Physiol Behav 86: 661–668.
34. Levin BE (2010) Developmental gene x environment interactions affecting systems regulating energy homeostasis and obesity. Front Neuroendocrinol 31: 270–283.
35. Plagemann A, Roepke K, Harder T, Brunn M, Harder A, et al. (2010) Epigenetic malprogramming of the insulin receptor promoter due to developmental overfeeding. J Perinat Med 38: 393–400.
36. Franke K, Harder T, Aerts L, Melchior K, Fahrenkrog S, et al. (2005) 'Programming' of orexigenic and anorexigenic hypothalamic neurons in offspring of treated and untreated diabetic mother rats. Brain Res 1031: 276–283.
37. Davidowa H, Plagemann A (2000) Decreased inhibition by leptin of hypothalamic arcuate neurons in neonatally overfed young rats. Neuroreport 11: 2795–2798.
38. Davidowa H, Plagemann A (2007) Insulin resistance of hypothalamic arcuate neurons in neonatally overfed rats. Neuroreport 18: 521–524.
39. Neitzke U, Harder T, Plagemann A (2011) Intrauterine growth restriction and developmental programming of the metabolic syndrome: a critical appraisal. Microcirculation 18: 304–311.
40. Landmann EM, Schellong K, Melchior K, Rodekamp E, Ziska T, et al. (2012) Short-term regulation of the hypothalmic melanocortinergic system under fasting and defined glucose-refeeding conditions in rats: A lasercapture microdissection (LMD)-based study. Neurosci Lett 515: 87–91.
41. Schmidt I, Schoelch C, Ziska T, Schneider D, Simon E, et al. (2000) Interaction of genetic and environmental programming of the leptin system and of obesity disposition. Physiol Genomics 3: 113–120.
42. Legro RS, Finegood D, Dunaif A (1998) A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. J Clin Endocrinol Metab 83: 2694–2698.
43. Levin BE, Hogan S, Sullivan AC (1989) Initiation and perpetuation of obesity and obesity resistance in rats. Am J Physiol 256: R766–R771.
44. Paxinos G and Watson C (1986) The Rat Brain in Stereotaxic Coordinates, second ed., San Diego: Academic Press.
45. Prüss H, Prass K, Gharni L, Milosevic M, Muselmann C, et al. (2008) Inducible nitric oxide synthase does not mediate brain damage after transient focal cerebral ischemia in mice. J Cereb Blood Flow Metab 28: 526–539.
46. Schmittgen T, Livak K (2008) Analyzing real-time PCR data by the comparative C_t method. Nat Protoc 3: 1101–1108.
47. Benoit SC, Air EL, Coolen LM, Strauss R, Jackman A, et al. (2002) The catabolic action of insulin in the brain is mediated by melanocortins. J Neurosci 22: 9048–9052.
48. Gundlach AL (2002) Galanin/GALP and galanin receptors: role in central control of feeding, body weight/obesity and reproduction? Eur J Pharmacol 440: 255–268.
49. Schwartz MW, Seeley RJ, Woods SC, Weigle DS, Campfield LA, et al. (1997) Leptin increases hypothalamic proopiomelanocortin (POMC) mRNA expression in the rostral arcuate nucleus. Diabetes 46: 2119–2123.
50. Woods SC, D' Alessio DA (2008) Central control of body weight and appetite. J Clin Endocrinol Metab 93: S37–S50.
51. Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, et al. (1995) Children born small for gestational age: do they catch up? Pediatr Res 38: 267–271.
52. Albertsson-Wikland K, Karlberg J (1994) Natural growth in children born small for gestational age with and without catch-up. Acta Paediatr 399: 64–70.
53. Crowther NJ, Cameron N, Trusler J, Gray IP (1998) Association between poor glucose tolerance and rapid post natal weight gain in seven-year-old children. Diabetologia 41: 1163–1167.
54. Dörner G, Grychtolik H, Julitz M (1977) Überernährung in den ersten drei Lebensmonaten als entscheidender Risikofaktor für die Entwicklung von Fettsucht und ihrer Folgeerkrankungen. Dt Gesundheitswesen 32: 6–9.
55. Dörner G, Rodekamp E, Plagemann A (2008) Maternal deprivation and overnutrition in early postnatal life and their primary prevention: historical reminiscence of an 'ecologic experiment' in Germany. Hum Ontogenet 2: 51–59.
56. Forsén T, Eriksson JG, Tuomilehto J, Osmond C, Barker DJP (1999) Growth in utero and during childhood among women who develop coronary heart disease: longitudinal study. BMJ 319: 1403–1407.
57. Ibánez L, Ong K, Dunger DB, de Zegher F (2006) Early development of adiposity and insulin resistance after catch-up weight gain in small-for-gestational-age children. J Clin Endocrinol Metab 91: 2153–2158.
58. Ong KKL, Ahmed ML, Emmett PM, Preece MA, Dunger DB, et al. (2000) Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ 320: 967–971.
59. Stettler N, Zemel BS, Kumanyika S, Stallings VA (2002) Infant weight gain and childhood overweight status in a multicenter, cohort study. Pediatrics 109: 194–199.
60. Plagemann A, Harder T (2005) Premature birth and insulin resistance (letter). N Engl J Med 352: 939–940.
61. Gan SK, Kriketos AD, Poynten AM, Furler SM, Thompson CH, et al. (2003) Insulin action, regional fat, and myocyte lipid: altered relationships with increased adiposity. Obes Res 11: 1295–1305.
62. Indulekha K, Anjana RM, Surendar J, Mohan V (2011) Association of visceral and subcutaneous fat with glucose intolerance, insulin resistance, adipocytokines and inflammatory markers in Asian Indians (CURES-113). Clin Biochem 44: 281–287.
63. Kotronen A, Yki-Järvinen H, Sevastianova K, Bergholm R, Hakkarainen A, et al. (2011) Comparison of the relative contributions of intra-abdominal and liver fat to components of the metabolic syndrome. Obesity 19: 23–28.
64. Shultis WA, Leary SD, Ness AR, Bain CJ, Emmet PM, et al. (2005) Does birth weight predict childhood diet in the Avon longitudinal study of parents and children? J Epidemiol Community Health 59: 955–960.
65. Ozanne SE, Hales CN (2004) Lifespan: catch-up growth and obesity in mice. Nature 427: 411–412.
66. Schwartz MW, Woods SC, Porte DJ, Seeley RJ, Baskin DG (2000) Central nervous system control of food intake. Nature 404: 661–671.
67. Leibowitz SF, Akabayashi A, Alexander JT, Wang J (1998) Gonadal steroids and hypothalamic galanin and neuropeptide Y: role in eating behaviour and body weight control in female rats. Endocrinology 139: 1771–1780.

5. Curriculum vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

6. Publikationsverzeichnis

I. Diplomarbeit

Schellong K. Einfluss von Capsaicin auf den postprandialen Stoffwechsel im weißen Fettgewebe. Eine Mikrodialyse-Studie. Institut für Ernährungswissenschaft der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Potsdam, 2003.

II. Originalarbeiten

1. Harder T, **Schellong K,** Plagemann A. Differences between meta-analyses on breastfeeding and obesity support causality of the association. (letter) Pediatrics 117 (2006) 987-988.
IF: 5,012

2. Harder T, Rodekamp E, **Schellong K,** Dudenhausen JW, Plagemann A. Birth weight and subsequent risk of type 2 diabetes: a meta-analysis. Am J Epidemiol 165 (2007) 849-857.
IF: 5,285

3. Harder T, **Schellong K,** Stupin J, Dudenhausen JW, Plagemann A. Where is the evidence that low birth weight leads to subsequent obesity? (letter) Lancet 369 (2007) 1859.

IF: 28,638

4. Neitzke U, Harder T, **Schellong K,** Melchior K, Ziska T, Rodekamp E, Dudenhausen JW, Plagemann A. Intrauterine Growth Restriction in a Rodent Model and Developmental Programming of the Metabolic Syndrome: A Critical Appraisal of the Experimental Evidence. Placenta 29 (2008) 246-254.
IF: 2,775

5. Plagemann A, Harder T, Brunn M, Harder A, Roepke K, Wittrock-Staar M, Ziska T, **Schellong K,** Rodekamp E, Melchior K, Dudenhausen JW. Hypothalamic proopiomelanocortin promoter methylation becomes altered by early overfeeding: an epigenetic model of obesity and the metabolic syndrome. J Physiol 587.20 (2009) 4963-4976.
IF: 4,764

6. Plagemann A, Roepke K, Harder T, Brunn M, Harder A, Wittrock-Staar M, Ziska T, **Schellong K,** Rodekamp E, Melchior K, Dudenhausen JW. Epigenetic malprogramming of the insulin receptor promoter due to developmental overfeeding. J Perinat Med 38 (2010) 393-400.
IF: 1,871

7. Landmann EM, **Schellong K,** Melchior K, Rodekamp E, Ziska T, Harder T, Plagemann A. Short-term regulation of the hypothalamic melanocortinergic system under fasting and defined glucose-refeeding conditions in rats: A lasercapture microdissection (LMD)-based study. Neurosci Lett 515 (2012) 87-91.
IF: 2,026

8. **Schellong K,** Schulz S, Harder T, Plagemann A. Birth weight and long-term overweight risk: systematic review and a meta-analysis including 643,902 persons from 66 studies and 26 countries globally. PLoS One 7 (2012) e47776.
IF: 3,730

9. **Schellong K,** Neumann U, Rancourt RC, Plagemann A. Increase of long-term 'diabesity' risk, hyperphagia, and altered hypothalamic neuropeptide expression in neonatally overnourished 'small-for-gestational-age' (SGA) rats. PLoS One 8 (2013) e78799.

IF: 3,730 (2012)

III. Übersichtsarbeiten

1. Harder T, Neitzke U, Melchior K, Rodekamp E, **Schellong K,** Ziska T, Dudenhausen JW, Plagemann A. The fetal origins hypothesis revised: animal studies and a meta-analysis. In: Zabransky S (ed.): SGA-Syndrom. Risiken für das cardio-pulmo-reno-vasculäre System. Conte Verlag, Saarbrücken (2006) 26-32.

2. Harder T, Rodekamp E, **Schellong K,** Dudenhausen JW, Plagemann A. Birth weight and subsequent risk of type 2 diabetes: a meta-analysis. In: Zabransky S (ed.): SGA-Syndrom. Risiken für das cardio-pulmo-reno-vasculäre System. Conte Verlag, Saarbrücken (2006) 32-37.

3. Harder T, **Schellong K,** Rodekamp E, Dudenhausen JW, Plagemann A. Perinatale Programmierung: Wird Adipositas im Mutterleib geprägt? Adipositas 1 (2007) 5-11.

4. **Schellong K,** Rodekamp E, Harder T, Dudenhausen JW, Plagemann A. Perinatale Prägung und lebenslange Krankheitsrisiken am Beispiel von Adipositas und Diabetes mellitus. Gynäkologe 41 (2008) 303-312.

5. Plagemann A, Harder T, Rodekamp E, **Schellong K,** Stupin J, Dudenhausen JW. Ernährung und frühe kindliche Prägung. In: Deutsche Gesellschaft für Ernährung (Hrsg.): Ernährungsbericht 2008. DGE Medienservice, Meckenheim (2008) 271-300.

6. Plagemann A, Harder T, **Schellong K,** Rodekamp E, Dudenhausen JW. Fetale Programmierung bei intrauteriner Milieusstörung - Grundlegende Mechanismen am Beispiel der Körpergewichts- und Stoffwechselregulation. Gynäkol Geburtshilfliche Rundsch 48 (2008) 215-224.

7. Harder T, **Schellong K,** Rodekamp E, Dudenhausen JW, Plagemann A. Birth weight and later risk of type 2 diabetes. In: Kiess W, Chernausek SD, Hokken-Koelega ACS (eds.): Small for gestational age. Causes and Consequences. Karger, Basel (2009) 60-72.

8. **Schellong K,** Rodekamp E, Harder T, Dudenhausen JW, Plagemann A. Geburtsgewicht, Gestationsdiabetes und Perinatale Programmierung. CME Prakt Fortbild Gynäkol Geburtsmed Gynäkol Endokrinol 3 (2009) 182-192.

9. Harder T, Rodekamp E, **Schellong K,** Dudenhausen JW, Plagemann A. Adipositas und perinatale Programmierung. In: Plagemann A, Dudenhausen JW (Hrsg.): Adipositas als Risiko in der Perinatalmedizin. Springer, München (2010) 72-81.

10. Plagemann A, Harder T, **Schellong K,** Schulz S, Stupin JH. Early postnatal life as a critical time window for determination of long-term metabolic health. Best Pract Res Clin Endocrinol Metab 26 (2012) 641-653.

IF: 4,912

Summe Impactpunkte: 62,743

Danksagung

Mein ganz außerordentlicher Dank gilt Herrn Prof. Dr. med. Andreas Plagemann, Leiter der Abteilung ‘Experimentelle Geburtsmedizin‘ der Klinik für Geburtsmedizin, für die Überlassung des Themas dieser Dissertation und seine umfangreiche wissenschaftliche Betreuung in jeder Phase dieser Arbeit. Seine wertvollen Anregungen sowie die kritischen und aufschlussreichen Diskussionen mit ihm haben zum Gelingen dieser Arbeit beigetragen.

Prof. Dr. med. Wolfgang Henrich, Direktor der Klinik für Geburtsmedizin, und Prof. Dr. med. Joachim W. Dudenhausen, emeritierter Direktor der Klinik, möchte ich für ihr Vertrauen und ihre Förderung als wissenschaftliche Mitarbeiterin der Klinik herzlich danken.

Ganz besonders möchte ich mich bei Herrn Dr. med. Thomas Harder, ehemaliger Mitarbeiter der Abteilung ‘Experimentelle Geburtsmedizin‘, für die Hilfestellungen bei der Auswertung der tierexperimentellen Untersuchungen und der Durchführung der Metaanalyse sowie seine kontinuierliche und engagierte Betreuung bedanken.

Bei Frau Dipl.-Vet. med. Kerstin Melchior und Herrn Dipl.-Biochem. Thomas Ziska möchte ich mich ganz herzlich für die engagierte und zuverlässige Zusammenarbeit bei der Durchführung der tierexperimentellen und hormonanalytischen Untersuchungen und ihre permanente Hilfsbereitschaft und uneingeschränkte Unterstützung bedanken.

Besonderer Dank gilt auch Frau Dr. med. Uta Neumann, Frau M. Sc. Emelie Landmann und Frau M. Sc. Sandra Schulz, die an den gemeinsamen Untersuchungen, Datenerhebungen und Datenauswertungen maßgeblich beteiligt waren.

Schließlich möchte ich allen nicht namentlich genannten Mitgliedern der Abteilung ‘Experimentelle Geburtsmedizin‘ für die gute Zusammenarbeit in den letzten Jahren danken.