Elevated glycemia and brain glucose utilization predict BDNF lowering since early life

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Abstract

Obesity and diabetes associate with neurodegeneration. Brain glucose and BDNF are fundamental in perinatal development. BDNF is related to brain health, food intake and glucose metabolism. We characterized the relationship between glycemia and/or brain glucose utilization (by [18]FDG-PET during fasting and glucose loading), obesity and BDNF in 4-weeks old (pre-obese) and 12-weeks old (obese) Zucker fa/fa rats, and their age-matched fa/+ controls. In 75 human infants, we assessed cord blood BDNF and glucose levels, appetite regulating hormones, body weight and maternal factors. Young and adult fa/fa rats showed glucose intolerance and brain hyper-utilization compared to controls. Glycemia and age were positively related to brain glucose utilization, and were negative predictors of BDNF levels. In humans, fetal glycemia was dependent on maternal glycemia at term, and negatively predicted BDNF levels. Leptin levels were associated with higher body weight and lower BDNF levels. Glucose intolerance and elevated brain glucose utilization already occur in young, pre-obese rats, suggesting that they precede obesity onset in Zucker fatty rats. Glycemic elevation and brain glucose overexposure predict circulating BDNF deficiency since perinatal and early life. Future studies should evaluate whether the control of maternal and fetal glycemia during late intrauterine development can prevent these unfavorable interactions.

Introduction

Diabetes, obesity and cognitive decline tend to occur in association. Brain glucose hypermetabolism has been shown to precede and predict the development of cognitive decline in humans.[1,2] It is also a feature of human obesity and diabetes, responding to weight loss.[3] We have previously shown that adult, hyperglycemic obese and diabetic Zucker rats are characterized by an elevated utilization of glucose in the brain.[4] These animals manifest a severe reduction in brain tissue volume,[5] thereby recapitulating the association of metabolic and neurodegenerative features seen in humans, in which brain glucose overexposure seems to be a common underlying factor.[2,3]

Brain-derived neurotrophic factor (BDNF) is an important neurotrophin, widely expressed throughout the central nervous system, and released by the brain into the circulation. It is involved in normal brain development and plasticity, learning and memory, aging and neurodegenerative disorders.[6–9] Recent studies indicate that a negative interaction occurs between impaired glucose tolerance and circulating BDNF levels,[10] which may contribute to explain the frequent

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association observed between diabetes and neurodegeneration.[11] In addition, a large body of evidence links diminished BDNF to the pathogenesis of obesity in humans and rodents.[10,12–14] Though the infusion of BDNF improves glucose metabolism[15] and ameliorates cognitive function and neurogenesis,[16–18] the pharmacological targeting of this pathway requires caution. A safer approach may be to identify and optimize mediators that indirectly promote BDNF production. The maintenance of adequate BDNF levels may be particularly important during early life, i.e. a time in which BDNF levels and brain plasticity are elevated, and diseases have not yet established.

The current study was undertaken to examine the relationship between body weight, glucose tolerance and BDNF in the Zucker rat model studied during early life or adulthood, i.e. before or after the onset of obesity, in fa/fa compared to fa/+ control rats. We used brain imaging by positron emission tomography (PET) to test the relationship linking brain glucose utilization and circulating glucose and BDNF levels. In human neonates, we explored the association between umbilical cord blood BDNF and glucose levels, appetite related hormones, birth body weight, and maternal factors.

Methods

Study in rats

Study design. We studied fa/fa Zucker pups (n = 22, age 26.4 ± 1.0 days) before the onset of obesity, and adult fa/fa Zucker rats (n = 21, age 11.7 ± 0.3 weeks) after development of the obese phenotype. Age-matched fa/+ Zucker pups (n = 17, 28.6 ± 0.8 days) and adult rats (n = 18, age 11.6 ± 0.3 weeks) were studied as controls. Adult animals were purchased from Charles Rivers Laboratories. Pups were produced by mating obese (fa/fa) males and heterozygous lean (fa/+) females. Since pups were indistinguishable based on body weight, group allocation was achieved by genotyping and plasma leptin measurements. Male pups were studied. In the adult groups, there were 19/2 (fa/fa) and 15/3 males/females (fa/+). Brain glucose utilization was determined after an overnight fasting period, by using PET in combination with ^{18}F-2-fluoro-2-deoxyglucose ([18F]FDG). Approximately half of the animals in each group were studied during fasting and the remaining during i.p. glucose loading. The experimental protocol was notified to the Ministry of Health (Dept. of Public Veterinary Health) in accordance with the D.L. 116/92, implementation of the directive EEC 609/86, regarding the protection of animals used for experimental and other scientific purposes, and is reported in compliance with the ARRIVE guidelines (Animal Research: Reporting in Vivo Experiments).

PET scanning protocol. Anaesthesia was induced and maintained by isofluorane (2.5 and 1.5% in oxygen, respectively). Femoral veins were surgically accessed and catheterized for tracer administration. PET imaging was performed with a dedicated small animal scanner (YAP(S)PET. I.S.E. s.r.l., Vecchiano, Italy). Animals were positioned supine on the scanner bed, and received an i.v. injection of [18F]FDG (21 ± 1 MBq in pups, 40 ± 1 MBq in adults) in a dilution volume of 100 μl (pups) or 200 μl (adults), followed by 100 μl of saline. A 20-min PET scan of the brain region was performed 60 min after injection. In the glucose loading studies, [18F]FDG injection was preceded (t ≅ −60 min) by the i.p. administration of 2 g/kg dextrose solution, and blood was frequently sampled by strip measurements via the tail tip to monitor and correct (via small administrations) circulating glucose to maintain constant glycemic values for one hour preceding the [18F]FDG scan and during the imaging period. Animals were euthanized by anesthetic overdose at the end of the experiments and blood was withdrawn for the assessment of insulin, BDNF and leptin levels. A sample of tail tissue was collected in young rats for genotyping.

Image analysis. Images were reconstructed using an iterative OSEM algorithm, as previously described.[4] Brain glucose utilization (reflecting transport and phosphorylation) was measured by drawing regions of interest (ROIs) on brain [18F]FDG PET images in areas involved in cognitive and metabolic control (i.e. frontal cortex, hippocampus, thalamus, striatum, putamen/caudate, pallidus, substantia nigra, hypothalamus, amygdala, olfactory bulb), as based on atlas guidance. Tissue [18F]FDG levels were divided by the dose of injected tracer per gram of animal to obtain standardized uptake values (SUV), representing an estimate of the regional brain glucose fractional extraction, and multiplied by blood glucose levels (SUV_g) to reflect brain glucose utilization rates. Whole brain glucose utilization was estimated as average of the above regional values.

Genotyping. Genotyping was performed in genomic DNA, by identifying the single or double restriction segments specific to homozygote (fa/fa) and heterozygous (fa/+) genotypes, as reported by Phillips et al.[19] and modified as described below.

Genomic DNA was purified from rat-tail (∼0.5 cm) using a commercially available kit (DNeasy blood and tissue kit, Qiagen s.r.l, Milan, Italy), according to manufacturer's instructions. DNA integrity was assessed by electrophoresis in 1% agarose gel, and DNA purity and quantity were given by value of 1.7–2.1 in the 260/280 nm optical density ratio (Infinite®

200 PRO, Tecan s.r.l. Milan, Italy). Genomic DNA was amplified in $40\,\mu l$ reactions containing $\sim 60\,ng$ of genomic DNA, with reported primers (5′- GTT TGC GTA TGG AAG TCA CAG-3′ and 5′-ACC AGC AGA GAT GTA TCC GAG-3′),[19] at a final concentration of $500\,nM$ each, and $20\,\mu l$ of GoTaq® Green Master Mix ready-to-use solution (Promega Italia s.r.l. Milan, Italy). The amplification protocol was 30 cycles at $95°C$ for $30\,s$, $58°C$ for $30\,s$ and $72°C$ for $2\,min$. PCR amplification resulted in a 1.8 kb product, whose integrity was assessed by electrophoresis in 1% agarose gel/TBE 0.5X (Sigma-Aldrich srl, Milan, Italy). PCR products were digested by incubating $17\,\mu l$ of PCR product with $1.5\,\mu l$ of restriction enzyme MspI (Promega Italia s.r.l. Milan, Italy) in a total volume of $20\,\mu l$ at $37°C$ overnight. MspI digestion of the 1.8 Kb fragment results in a single restriction site (950 bp fragment) or in two restriction sites (1100 bp and 950 bp fragments) for the obese homozygous and the lean heterozygous rat, respectively.

Study in humans

Study design. The study included 75 neonates and their mothers. Mothers were recruited either at the beginning or at the end of pregnancy. Maternal body mass index (BMI) and fasting plasma glucose and insulin measurements were available at the beginning of pregnancy, and/or at the time of glucose tolerance testing, and/or at delivery, according to sample sizes shown in Table 1. Body weight and height in neonates at the time of birth were used to estimate the ponderal index (PI), computed as ratio of weight to the third power of height (kg/m^3). Umbilical cord blood was drawn after cord dissection to assess neonatal BDNF, glucose, insulin, leptin, and PYY levels. The human study was approved by the Local Ethical Committee (Comitato Etico per la sperimentazione clinica dei medicinali dell'Azienda USL1 di Massa e Carrara, CESM, project number 309 and 394), which complies with the Helsinki Declaration 1964 (and subsequent amendments), and the Oviedo Convention (1997, ratified by Law 28/03/2001, n. 145). Both parents gave written informed consent before study initiation.

Measurement of circulating biomarkers

Human and animal blood samples were centrifuged for 10 min at 3000 rpm, and plasma or serum was stored at $-80°C$. In human samples, plasma glucose and insulin levels were analysed by routine enzymatic method (Synchron CX9 Pro Beckman Coulter, Inc, Brea, CA, USA and Abbott s.r.l. Italy, respectively); BDNF was measured by sandwich enzyme immunoassay (ChemiKine™ Chemicon International Inc, MA,

Table 1. Maternal and neonatal characteristics in the human study.

	N	
Mother		
Age (years)	75	33.2 ± 0.6
BMI (kg/m²) 1st trim	75	24.3 ± 0.6
BMI (kg/m²) 2nd trim	58	28.0 ± 0.6
BMI (kg/m²) 3rd trim	69	29.0 ± 0.6
Fasting glucose levels (mmol/l) 1st trim	64	4.5 ± 0.1
Fasting glucose levels (mmol/l) 2nd trim	65	4.6 ± 0.1
Fasting glucose levels (mmol/l) 3rd trim	40	5.1 ± 0.2
Fasting insulin levels (mU/l) 2nd trim	29	9.0 ± 0.7
Fasting insulin levels (mU/l) 3rd trim	44	12.1 ± 1.5
Parity (1, 2, >2, including current child)	75	40/31/4
Gestational diabetes (no/yes)	75	57/18
Mode of delivery (spontaneous/C-section)	75	48/27
Neonate		
Gestational age (days)	75	277 ± 1
Ponderal index (kg/m³)	75	26.8 ± 0.3
Cord glucose levels (mmol/l)	70	3.8 ± 0.2
Glycemia 2 h after birth (mmol/l)	67	3.4 ± 0.1
Cord insulin levels (mU/l)	72	4.8 ± 0.4
Cord leptin levels (ng/ml)	75	20.9 ± 2.2
Cord PYY levels (ng/ml)	75	0.29 ± 0.02
Cord BDNF (ng/ml)	75	12.9 ± 0.09

USA), and circulating levels of leptin, and PYY were measured by multi-analyte panels based on Luminex® xMAP® technology (Milliplex map kit, CAT N# HMHMAG-34K and HPTP2MAG-66K, EMD Millipore Corp., MA, USA). In rats, glycemia was monitored by glucometer (LifeScan OneTouch®, Johnson and Johnson Medical SpA, Rome, Italy), throughout the study session, and values above the measurement range were set at the maximum of that range, circulating levels of leptin, insulin, and BDNF were measured by multi-analyte panels based on Luminex® xMAP® technology (Milliplex map kit, CAT N# RMHMAG-84K and RPTMAG-86K, EMD Millipore Corp., MA, USA). All procedures were carried out according to manufacturer's instruction.

Statistical analysis

Data are presented as mean $\pm$ SEM. Image and biochemical analyses were done blindly with respect to genotype (animal study) or neonatal characteristics (human study). Age-matched groups were compared by using two-tailed analysis of variance. The Pearson correlation coefficient was used to evaluate the strength of linear correlations. Differences between groups were

regarded as statistically significant when the *p* value was ≤0.05.

Results

Animal study

No differences in body weight were observed between fa/+ (68 ± 5 g) and fa/fa rats (61 ± 5 g) at the age of four weeks. Instead, adult (12 week old) fa/fa animals were 39% heavier than age-matched controls (388 ± 9 vs. 280 ± 10 g, *p* < 0.0001). As expected, leptin values were higher in fa/fa compared to respective controls (pups: 10031 ± 1135 vs. 2283 ± 287 pg/ml, *p* < 0.0001; adults: 21999 ± 2967 vs. 948 ± 156 pg/ml, *p* < 0.0001). Leptin levels declined by 59% in adults compared to pups in fa/+ rats (*p* = 0.0004), whereas they rose by 119% in fa/fa rats (*p* = 0.002).

In both age groups, fa/fa Zucker rats were glucose intolerant, as compared with age-matched fa/+ animals (Figure 1). Hyperglycemia was present during the glucose load in young fa/fa animals, in spite of normal body weight, and during both fasting and glucose loading in adult obese fa/fa animals compared to respective control groups. Consequently, glucose levels were higher in glucose loaded than fasted rats, except for the adult fa/fa group in which no difference occurred. In fa/+ animals, insulin levels were lower in adults than pups, due to physiological aging, but this age-related pattern was not observed in fa/fa rats (Figure 1), as adult obese rats showed fasting hyperinsulinemia. Compared to respective control rats, insulin levels were four times higher in fa/fa adults during fasting, and 70% lower in fa/fa pups during glucose loading. In glucose loading studies, insulin levels were 2–3 folds higher than in fasting studies in fa/+ groups, whereas no such β-cell response was seen in fa/fa groups.

Brain glucose utilization (Figure 2) during glucose loading was ∼50% higher in 4-week and 12-week-old fa/fa versus age-matched fa/+ animals. In adult fa/fa animals, brain glucose utilization was severely increased also during fasting (+100%) compared to adult fa/+ rats. Thus, the physiological difference in brain glucose utilization occurring during glucose loading versus fasting conditions was absent in this group. Brain glucose utilization was positively predicted by adult age, fa/fa genotype and glucose loading (Figure 3). In turn, brain glucose utilization was a strong negative predictor of circulating BDNF levels together with concurrent glycemia, and age (Figure 3). Relationships between BDNF and genotype per se (*p* = 0.11), or leptin levels (*p* = 0.51) or body weight (age-adjusted, *p* = 0.75) were not significant.

Human study

Metabolic characteristics of mothers and neonates are given in Table 1. Maternal glycemia at term was significantly and positively correlated with neonatal glycemia (r = +0.66, *p* < 0.0001), whereas maternal glycemia in the first and second trimesters was not (*p* = 0.24–0.25). In turn, umbilical cord glycemia was a strong negative predictor of cord BDNF levels (Table 2). Cord leptin was more weakly associated, whereas neonatal body weight (*p* = 0.96) or ponderal index (*p* = 0.39) were not correlated with BDNF levels at birth. Body weight was significantly associated with leptin levels (r = 0.27, *p* = 0.02). Maternal factors, including BMI, frequency of gestational diabetes, parity, age, mode of delivery (C-section vs.
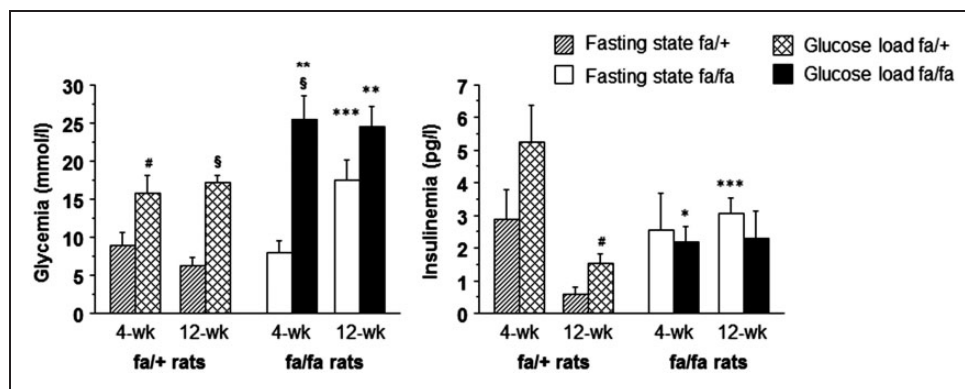

Figure 1. Glucose levels are shown in the *left panel*, documenting post-glucose load hyperglycemia in 4-week-old (non-obese) fa/fa rats, and fasting and post-glucose load hyperglycemia in adult 12-week-old (obese) fa/fa rats compared to respective controls. Insulin levels (*right panel*) demonstrate fasting hyperinsulinemia in adult fa/fa rats, and the lack of β-cell response to hyperglycemia in all fa/fa groups. ***p ≤ 0.003, **p = 0.02 and *p = 0.04 vs. age-matched fa/+ controls; #p < 0.03, §p ≤ 0.0005 vs. fasting state.
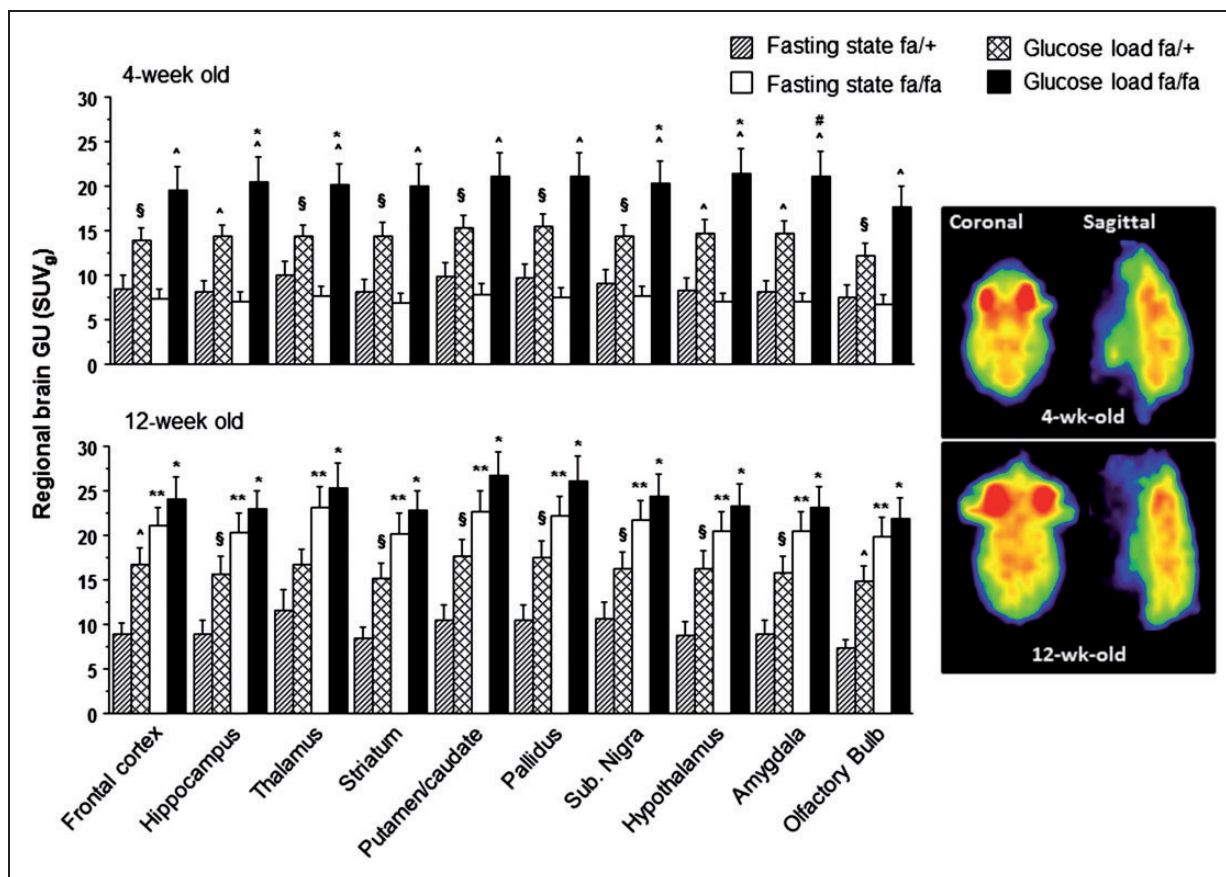
Figure 2. Regional brain glucose utilization (GU) rates (*top panel*) show hypermetabolic regions during glucose loading in fa/fa pups (*top panel*), and during both fasting and glucose loading in fa/fa adults (*bottom panel*) compared to respective controls. A significant increase in GU occurs in all brain regions during glucose loading compared to fasting in all groups, except for adult fa/fa rats. Representative examples of 4-week and a 12-week-old rats are shown on the right; the color scale ranges from red (higher activity) to orange, yellow, green and blue (lower activity). $**p \leq 0.002$, $*p < 0.05$ and $^{\#}p = 0.052$ vs. age-matched fa/+ rats; in pups, the remaining regions were close to significance ($p = 0.6–0.9$); $^{\wedge}p < 0.005$ and $^{\S}p \leq 0.05$ vs. fasting state.

Discussion

Our data in rats suggest that glucose intolerance and cerebral glucose utilization are strong negative predictors of BDNF levels. In human neonates, we found a similar negative relationship between cord blood BDNF and glucose levels, and the latter seemed determined by maternal glucose levels at term.

The animal study was conducted in young and adult rodents to examine the relationship linking BDNF to glucose tolerance or obesity. Young fa/fa animals were normal weight compared to age-matched controls, although genetically committed to develop obesity. The pre-obesity phase was characterized by glucose intolerance, apparently due to insufficient insulin release in response to glucose loading. Compared to

young non-obese fa/fa rats, the occurrence of obesity in adult fa/fa animals did not further affect insulinemia after glucose loading. This implicates β-cell glucose insensitivity in the development of glucose intolerance in this animal model, in keeping with data obtained in isolated perfused pancreas, showing that no insulin secretory response to incremental glucose stimulation in the upper glycemic range occurs in fatty Zucker rats.[20] Instead, the onset of obesity in our adult rats resulted in fasting hyperglycemia and fasting hyperinsulinemia, which reflect hepatic insulin resistance.

Our data support the notion that age is one main negative regulator of central BDNF production.[21] As seen in relation to glucose intolerance, obesity per se did not explain BDNF concentrations, whereas our results suggest that circulating levels of BDNF strongly relate to plasma glucose concentrations. We reported an inverse association linking chronic glycemia and BDNF. In addition, we showed that BDNF levels
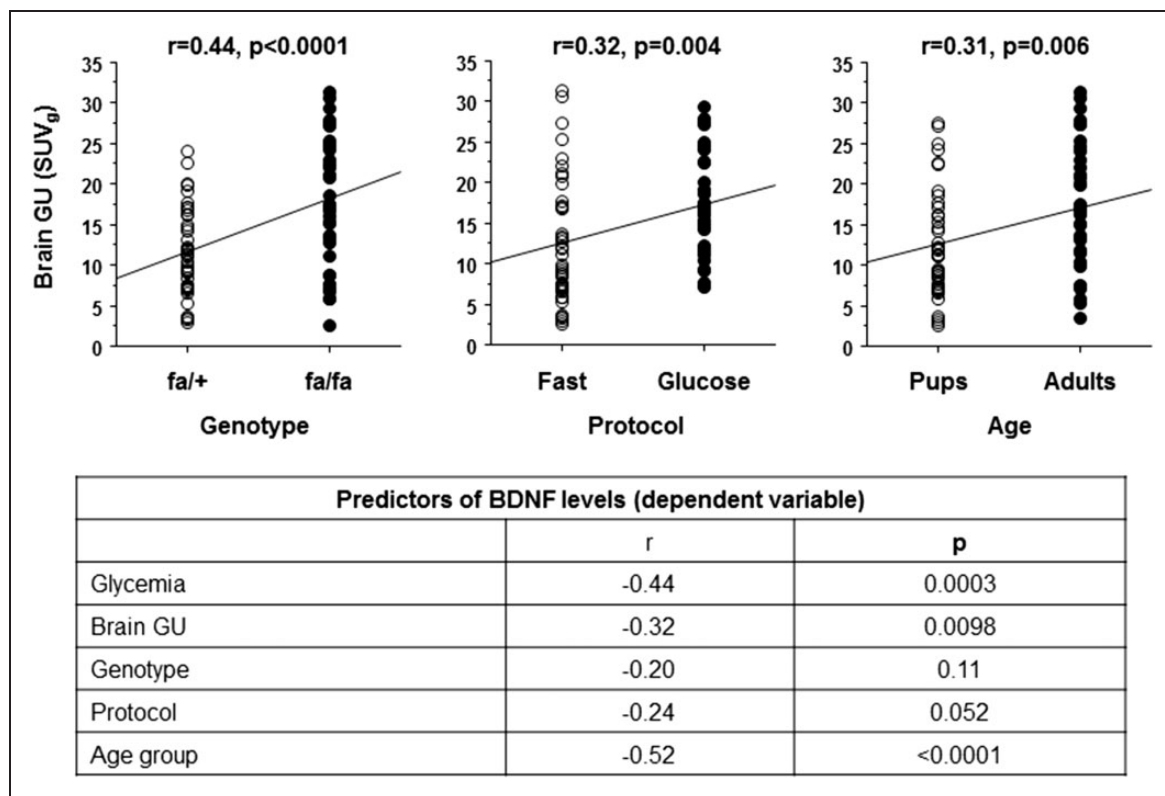
Predictors of BDNF levels (dependent variable)		
	r	p
Glycemia	-0.44	0.0003
Brain GU	-0.32	0.0098
Genotype	-0.20	0.11
Protocol	-0.24	0.052
Age group	-0.52	<0.0001

Figure 3. Across groups, brain glucose utilization (GU, *top panels*) shows positive dependence on fa/fa genotype, glucose loading protocol and adult age. Data in the *bottom panel* indicate that brain GU, glycemia (primary regulator of GU) and age group, rather than genotype per se, were strong negative predictors of BDNF levels in rats.

Table 2. Predictors of cord BDNF levels (dependent variable).

	r	p
Cord glycemia	−0.42	0.0003
Cord leptinemia	−0.28	0.01
Cord PYY	+0.21	0.08
Ponderal index	−0.10	0.39
Maternal glycemia	−0.29	0.08

were lower during the glucose loading than fasting protocol, supporting a suppressive effect of acute glucose loading on circulating BDNF concentrations. The current fa/fa model is characterized by the genetic lack of leptin receptors, and leptin infusion has been shown to stimulate BDNF expression.[13,14,22] However, different from an exogenous infusion, leptin secreted by adipose tissue in this animal model reflects the combined degrees of adiposity and leptin resistance due to the lack of leptin receptors, and the balance between these factors did not result in a significant correlation between leptin and BDNF levels, nor was BDNF dependent on genotype per se.

An elevated brain glucose utilization in response to insulin has been observed in human subjects with obesity and with impaired glucose tolerance.[3,23] Extending this observation to daily-life conditions, we previously reported a chronic (fasting and post-glucose load) state of brain glucose hyper-utilization in adult obese fa/fa rats, which was reinforced in Zucker diabetic fatty (ZDF) rats.[4] The present study is consistent with the above evidence in adult models. However, studies in adult humans and rats with the metabolic syndrome do not allow disentangle the interdependency of chronic hyperglycemia and obesity in regulating brain glucose utilization. One novelty of the present study was to demonstrate that brain glucose utilization was 50% higher during glucose loading before the onset of weight gain in glucose intolerant young fa/fa rats. Brain glucose hypermetabolism has been recently recognized as a pre-eminent feature of neurodegenerative diseases.[2] BDNF is implicated in the pathogenesis of these diseases, and its deficiency is proportional to the severity of cognitive impairment in humans.[7] BDNF levels are also reduced in the brain of diabetic mice and in the circulation of diabetic humans.[10,11,24] In our study, brain glucose utilization was a strong negative

predictor of BDNF levels, independent of body weight. In humans, hyperglycemic, and not euglycemic hyper-insulinemia leads to a decline in BDNF release by the brain, as directly measured by arterial-venous catheter-ization.[10] We observed a correlation between glucose levels and brain glucose utilization. Though we may speculate that brain glucose utilization mediates the effects of hyperglycemia on BDNF levels, correlations do not imply causality, and studies addressing the effects of hyperglycemia on cerebral glucose phosphor-ylation and subsequent signaling in vivo are needed to support this interpretation.

Brain metabolism and BDNF are also implicated in the control of eating behavior and body weight. Meal or glucose ingestion inhibit, whereas fasting stimulates appetite. The expression of BDNF in the brain is sup-pressed by fasting and stimulated by refeeding.[13] Glycemic excursions are also important in the cycling between fasting and food seeking behavior,[25] and the hypothalamic unresponsiveness to a glucose load has been suggested to explain continuing appetite and over-eating in obese people.[26] One interesting difference between our young pre-obese and adult obese fa/fa ani-mals was that the former maintained an up-regulatory excursion in brain utilization during glucose loading compared to fasting, whereas brain glucose utilization was constantly high and did not change in fasting versus glucose loaded conditions in obese adult rats. It is tempting to speculate that the preservation of metabolic flexibility in the brain of our young fa/fa rats may contribute to maintain their normal weight, as opposed to the loss of metabolic excursion seen in the brain of adult fa/fa rats, which may stimulate hyperphagia. Higher glycemic and brain utilization levels achieved post-glucose loading in young fa/fa than control rats, suppressing BDNF, seemed to pre-cede and may contribute to the establishment of weight gain, in agreement with the suggested involvement of BDNF in the inhibition of appetite.[13,14]

We conducted an explorative analysis in human neo-nates. Human findings from the current study provided support to the concepts derived in the animal model. First, BDNF values observed in our neonates were higher than reported in healthy adults[27] and elderly people,[9] in agreement with the strong age-dependency seen in our rats. This is also consistent with post-mortem human lifespan brain findings on BDNF expression,[28] showing peak values in the neonatal period, underlying the importance of BDNF in early cognitive development. Second, the inverse association between glucose and BDNF levels in neonatal plasma was strong. Notably, both the tightness (r = −0.44 vs. −0.42) and the level of significance of this correlation (p = 0.0003 vs. 0.0003) were identical in the animal and human model. Glycemia in newborns was predicted by

maternal glycemia occurring at the end of pregnancy, not in the first or second trimesters, indicating a critical time window in which the control of maternal glycemia may be protective of brain health in the upcoming child. In fact, the last weeks of pregnancy witness a many-fold increase in brain size, and the delineation of brain structures and functions. Also, occurring during this period is most of body growth and adipose tissue hypertrophy. Interestingly, we found that cord leptin, reflecting adiposity, was negatively correlated with BDNF levels, and positively related to body weight in infants. Again similar to the animal model, we did not observe a direct relationship between BDNF levels in cord blood and ponderal index in newborns. Though associations do not demonstrate causality, it is important to underline that BDNF is a limiting factor for the action of leptin in the brain.[14] Thus, one possi-bility to explain our finding is that lower BDNF values may result in brain leptin resistance, leading to a com-pensatory expansion in adipose tissue to release more leptin. From a clinical standpoint, it is of note that our human findings mostly referred to physiological mater-nal and neonatal conditions, and were not dependent on gestational diabetes. Thus, our data prompt for a reappraisal of the optimal maternal glycemic range, specific to late gestation, to protect neonatal brain development from BDNF lowering.

This study had some limitations. Our comparisons were cross-sectional, as in the animal study we opted to examine two very distinct conditions (pre-obesity and overt obesity), and the current sample collection pre-cluded the possibility to study the same animals repeat-edly. Cross-sectional comparisons and correlations only allowed us to postulate causation, and speculate on disease progression. Though young and adult rats had the same genetic background, we recognize that longitudinal observations and the examination of inter-mediate age-points would be required to provide time-dependent trajectories. Similarly, follow-up studies in our human neonates will evaluate whether cord BDNF and glycemia may predict subsequent body growth, eating behavior and cognitive development.

In conclusion, our data indicate that glucose intoler-ance and elevated brain glucose utilization are already present in young, pre-obese rats, suggesting that they precede the onset of obesity in Zucker fatty rats, and are negatively related to BDNF levels. This interaction may be most incisive during early brain development, when BDNF levels were high. In line with animal data, neonatal glycemia was a strong negative predictor of BDNF levels in cord blood, and it was dependent on maternal glucose levels at term. Lower BDNF was also accompanied by higher cord leptin, relating to body weight. Overall, these findings call for action to evalu-ate whether the control of maternal and fetal glycemia

during late intrauterine development can prevent its unfavorable interaction with BDNF.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' contributions

Guzzardi MA: coordination, data collection, genotyping, and blood assays in the animal and human studies; Sanguinetti E: co-coordination and data collection in the human study; Burchielli S: responsibility for animal safety and preparation; Bartoli A and Panetta D: image acquisition and reconstruction; Kemeny A: data collection in the human study; Salvadori PA: coordination and financing of imaging facilities and radiopharmacy, synthesis and provision of PET tracers; Iozzo P: project design and supervision, recipient of the Tuscany Region grant (Innovation in Medicine 2009) financing the study, analyses of images and data, and manuscript writing. All authors revised and accepted the manuscript in its current form.

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