British Journal of Pharmacology

DOI:10.1111/bph.13284
www.brjpharmacol.org

RESEARCH PAPER

Chronic intermittent toluene inhalation in adolescent rats results in metabolic dysfunction with altered glucose homeostasis

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Received
26 March 2015
Revised
9 August 2015
Accepted
10 August 2015

BACKGROUND AND PURPOSE

Abuse of toluene-containing inhalants is an increasing public health problem, especially among adolescents. Abuse during adolescence is associated with emaciation, while industrial exposure leads to altered glycaemic control suggesting metabolic instability. However, the relationship between adolescent inhalant abuse and metabolic dysfunction remains unknown.

EXPERIMENTAL APPROACH

To model human abuse patterns, we exposed male adolescent Wistar rats [postnatal day (PND) 27] to chronic intermittent inhaled toluene (CIT, 10 000 ppm) or air (control) for 1 h·day^{-1}, three times a week for 4 weeks. Feeding and body composition were monitored. After 4 weeks, circulating metabolic hormone concentrations and responses to a glucose tolerance test (GTT) were measured. Dietary preference was measured by giving animals access to either a 'western diet' plus standard chow (WC + SC) or standard chow alone during 4 weeks of abstinence. Metabolic hormones and GTT were subsequently measured.

KEY RESULTS

Adolescent CIT exposure significantly retarded weight gain, altered body composition, circulating metabolic hormones and responses to a GTT. While reduced body weight persisted, responses to a GTT and circulating hormones appeared to normalize for animals on standard chow following abstinence. In CIT-exposed WC + SC rats, we observed impaired glucose tolerance associated with altered metabolic hormones. Analysis of hypothalamic genes revealed differential expression profiles in CIT-exposed rats following both the exposure period and abstinence, suggesting a central contribution to inhalant-induced metabolic dysfunction.

CONCLUSION AND IMPLICATIONS

CIT exposure during adolescence has long-term effects on metabolic function, which may increase the risk of disorders related to energy balance and glycaemic control.

Abbreviations

Agrp, agouti-related protein; ARC, arcuate nucleus; CIT, chronic intermittent toluene; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; GTT, glucose tolerance test; Hprt1, hypoxanthine phosphoribosyltransferase 1; MCP-1, monocyte chemotactic protein-1 (also known as CCL2); PND, postnatal day; PP, pancreatic polypeptide; PVN, paraventricular nucleus; qPCR, real-time PCR; RM, repeated measures; SC, standard chow; WC, western chow

Tables of Links

TARGETS	
GPCRs[a]	**Catalytic receptors**[b]
Ghrelin receptor (Ghsr)	InsR (Insr)
	Leptin receptor (Lepr)

LIGANDS		
Agrp	Glucagon	MCP-1 (CCL2)
Amylin	Glucose	Neuropeptide Y (NPY, Npy)
C-peptide	IL-6	Pancreatic polypeptide (PP)
CRH (Crh)	Insulin	POMC (ACTH; Pomc)
GIP	Leptin	PYY
GLP-1		Toluene

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b]Alexander *et al.*, 2013a,b).

Introduction

The abuse of inhaled chemical vapours that contain volatile solvents, such as toluene, is a significant public health concern, especially among adolescent and indigenous populations who represent over 60% of the abusive population (Cairney *et al.*, 2002; Lubman *et al.*, 2006). Consequently, inhalants are typically one of the first classes of 'drugs' that young people misuse (AIHW, 2011) and is associated with long-term adverse outcomes (Johnson *et al.*, 1995; White, 2001). Furthermore, in Australia, the popularity of inhalant misuse has recently increased by 23%, while both alcohol and tobacco use has decreased (AIHW, 2011), suggesting significant growth of this issue in society.

In humans, chronic abuse of inhalants is associated with weight loss (Glaser and Massengale, 1962) and emaciation (Ryu *et al.*, 1998), plus forms of disordered eating (Pisetsky *et al.*, 2008) and altered dietary preference (Wang *et al.*, 1998) suggesting underlying changes in energy homeostasis. This is further supported by reports indicating that chronic exposure to inhalants alters circulating levels of metabolic hormones, in particular those involved in regulating glycaemic control. For example, male industrial workers chronically exposed to low levels of volatile solvents including toluene display increased cholesterol and altered fasting glucose and insulin levels (Won *et al.*, 2011). These individuals are insulin resistant, with impaired glucose metabolism the first step towards a diabetic phenotype (Won *et al.*, 2011). Together, this suggests that inhalants result in severe metabolic alterations including those involved in glucose metabolism. However, the link between adolescent inhalant misuse and risk for adult onset disorders related to altered metabolic function and glycaemic control in high-risk populations is yet to be investigated.

Inhalant-induced metabolic changes may be influenced by numerous factors, including changes to central or systemically mediated metabolic signalling, energy balance and/or organ function. However, the underlying mechanisms mediating inhalant induced-metabolic dysfunction are likely to be complex and multifactorial. Case studies have reported hyperchloraemic metabolic acidosis, hypokalaemia (Camara-Lemarroy *et al.*, 2012), increased alkaline phosphatase levels and proteinaemia, kidney dysfunction (Brautbar, 2004), renal tubular acidosis (Carlisle *et al.*, 1991), rhabdomyolysis (Streicher *et al.*, 1981) and liver dysfunction (Ayan *et al.*, 2013) in individuals that misuse inhalants, suggesting that systemic organ dysfunction may play a contributing factor. There is also clear evidence that inhalants affect the CNS (Yucel *et al.*, 2008; Dick *et al.*, 2014). Hypothalamic syndrome has been reported in human abusers (Teelucksingh *et al.*, 1991) as well as altered temperature regulation, adipsia and cranial diabetes insipidus – all characteristics typical of hypothalamic dysfunction (Carmel, 1980). Exposure to toluene in rats affects the number of hypothalamic neuropeptide Y (NPY) neurons (Shen *et al.*, 1992), which regulate food intake and fat storage, and decreases protein expression for both NPY and galanin specifically in the paraventricular nucleus (PVN) and arcuate nucleus (ARC) (Moron *et al.*, 2004). Together, these studies suggest a central component is likely to be involved in toluene-induced metabolic dysfunction.

Due to the high prevalence of inhalant abuse, especially in adolescent populations, the observed inhalant-induced metabolic dysfunction in human abusers, including altered glycaemic control, warrants further investigation. Furthermore, there are scant data pertaining to the persistence of inhalant-induced metabolic dysfunction even if individuals maintain abstinence. Therefore, the present study aimed to investigate the relationship between inhalant-induced metabolic dysfunction and altered glycaemic control following chronic intermittent exposure to the volatile solvent toluene (methyl benzene) at an abuse concentration [chronic intermittent toluene (CIT), 10 000 ppm] during adolescence in a rodent model, where adolescence in rodents ranges from postnatal day (PND) 21 to PND 60 (Andersen, 2003). We examined toluene in isolation as it is found in many household products including glues and aerosols and has high abuse potential due to its ability to modulate reward pathways (Riegel *et al.*, 2007). We also investigated the persistence of such alterations following a period of sustained abstinence. As inhalants have been suggested to alter dietary preference (Wang *et al.*, 1998) and the potential of diet to influence glycaemic control (Won *et al.*, 2011), we also investigated the same

parameters when animals were given access to a 'western diet' (high fat, sugar and sodium) in association with standard rodent chow. This diet was presented during abstinence only to reduce the potential interaction between diet and toluene during the exposure period. We hypothesized that adolescent CIT would affect the control of energy balance, thus altering metabolic function and glycaemic control. We also hypothesized that this would involve changes to centrally mediated processes involved in energy regulation. Finally, we hypothesized that these effects would be perpetuated when the animals were also given access to a western diet during abstinence. Our study determined that CIT exposure during adolescence has long-term effects on metabolic function, which may increase the risk of disorders related to energy balance and glycaemic control, such as diabetes.

Methods

Animal welfare and ethical statement

Adolescent male Wistar rats ($n = 72$, approximately PND 24) were obtained from the Australian Resources Centre (Perth, WA, Australia). Rats were housed singly to permit monitoring of food and water intake, maintained on a 12 h light/dark cycle with food and water *ad libitum*. Rats were acclimatized for 3 days before experimentation. All experimental procedures used were as humane as possible and were approved by the Florey Institute of Neuroscience and Mental Health ethics committee (March 2013; project 13-021). Experiments were performed in accordance with the Prevention of Cruelty to Animals Act 1986 under the guidelines of the Australian National Health and Medical Research Council Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia and are in compliance with institutional, national and international guidelines. All the animal experiments comply with the recommendations of the ARRIVE guidelines (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010).

Toluene inhalation

Exposure to 10 000 ppm vapourized liquid toluene (1.08389, purity 99.8%, Merck, Bayswater, Vic., Australia) was conducted as previously described (Duncan *et al.*, 2012; Dick *et al.*, 2014). Briefly, exposure was conducted in dynamic chambers constructed using toluene-resistant materials (Alternative Plastics Pty. Limited, North Melbourne, Vic., Australia) and fittings (Swagelok, Broadmeadows, Vic., Australia) with each single unit (36.6 cm wide × 19.5 cm high × 17.2 cm deep) composed of two chambers (17.6 cm wide × 16.5 cm high × 16.4 cm deep), which both housed an individual rat throughout the exposure. The concentration (10 000 ± 100 ppm) of toluene was verified using a calibrated inline gas chromatography system (Shimadzu Corporation, Kyoto, Japan). Chambers of similar design but exposed to room air were utilized for control animals (0 ppm exposure).

Rats were acclimatized to the laboratory for 1 h before exposure to toluene or air during which time their body weights were recorded. Rats were randomly assigned to inhale either air ($n = 36$) or toluene (10 000 ppm, $n = 36$) for 1 h·day^{-1}, 3 days·week^{-1} (Monday, Wednesday and Friday), for 4 weeks (PNDs 27–52) in three separate cohorts (Figure 1). This exposure paradigm was employed to reflect the human pattern of toluene abuse at a relevant concentration (Lubman *et al.*, 2008) where the sensitivity to toluene is approximately equivalent in both species (Benignus *et al.*, 2007). After 1 h, rats were placed back into their home cages and isolated from other rats for 1 h to avoid olfactory stimulation by toluene scent on the fur before being returned to their holding rooms. Exposures were conducted at room temperature (~21°C) under normal lighting, and each rat was exposed at the same time each day (2–4 h into the light cycle). Behavioural effects of this exposure paradigm have been reported previously (Dick *et al.*, 2014; Duncan *et al.*, 2014).

Food and water intake

Throughout the exposure (4 weeks) and subsequent abstinence (4 weeks) periods, food and water intake were measured on Monday, Wednesday and Friday, and daily intake was calculated for rats in cohort 1 (Figure 1A). To examine the effects of CIT on dietary preference, a subset of rats (air $n = 10$, CIT $n = 10$) was given free access to western diet chow [WC, high in fat (>20%), sodium (~12 mg·g^{-1}) and sugar (~20 kj·g^{-1}); SF13-036, Specialty Feeds, Perth, WA, Australia; Ong and Muhlhausler, 2011] in addition to standard chow (SC) throughout abstinence (PNDs 57–91). Rats given access to both WC and SC are hereafter referred to as WC + SC. To examine a preference for palatable rewards following abstinence from CIT, at PND 96, a separate cohort of animals (cohort 2, $n = 10$ per group, Figure 1B) was given free access to both water and saccharin (0.1% w v^{-1}) for 48 h, and fluid consumption was recorded.

Echo-MRI

Body composition was examined twice within 2 min using an Echo-MRI Analyser (Echo Medical Systems, Houston, TX, USA). Fat, lean muscle and total water levels were measured at baseline (before any exposures, PND 27, $n = 20$ per group), on the final day of exposure (PND 52, $n = 20$ per group) and during the final week of abstinence (PND 86; SC, $n = 10$ per group; WC + SC, $n = 10$ per group, Figure 1A). Values are presented as a mean of two readings.

Circulating metabolic hormones

To analyse circulating hormone levels in fasted (12 h food deprivation, $n = 8$–10 per group) and nonfasted ($n = 8$–10 per group) conditions, plasma was collected 72 h following the exposure period (PND 55), to permit sufficient time for toluene to be cleared from tissues, and following abstinence (fasted, $n = 6$–10 per group, PND 83; nonfasted $n = 6$–10 per group, PNDs 90–91, Figure 1A) conditions. Samples were collected 2–4 h into the light cycle, and the order randomized between air and CIT-exposed rats to control for potential circadian effects. Approximately 300 μL of tail blood was collected into BD800 blood metabolic preservation tubes (BD, North Ryde, NSW, Australia), centrifuged at 8400 *g* (Clement GS200) at 4°C, and the resulting plasma assayed for amylin, C-peptide, gastric inhibitory polypeptide (GIP), glucagon-like peptide-1 (GLP-1), glucagon, IL-6, insulin, leptin, monocyte chemotactic protein-1 (MCP-1 also known as CCL2), pancreatic polypeptide (PP) and peptide YY (PYY) using a Millipex Map rat panel (Cardinal Bioscientific, New Farm, QLD, Australia); all values are in pg mL^{-1}. This is a commercially available multiplex metabolic assay designed to capture

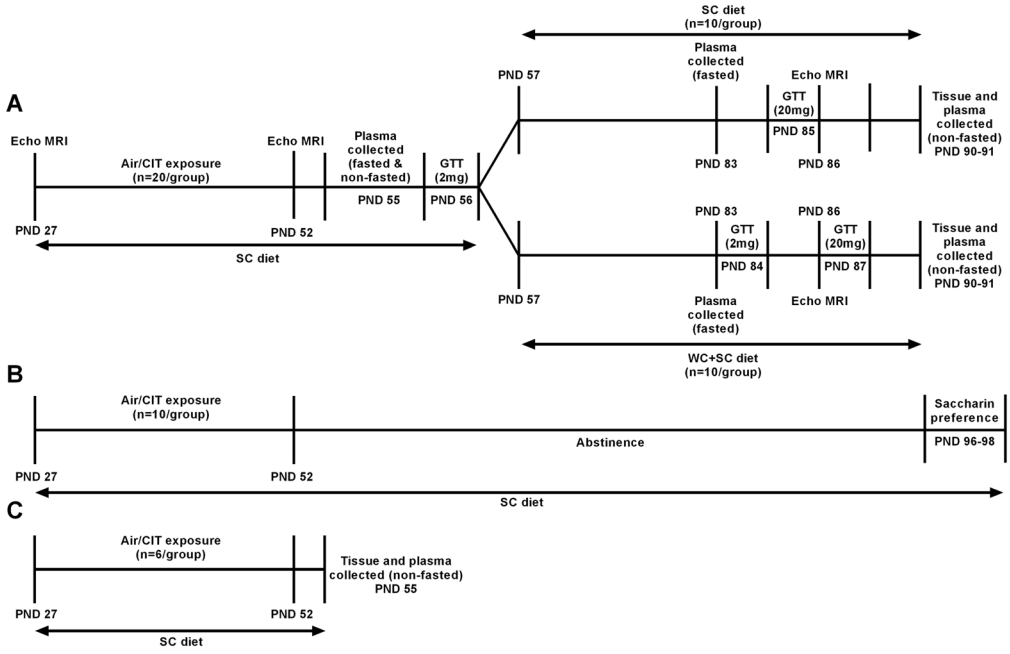

Figure 1

Experimental timeline. Overview of experiments conducted in cohort 1 (A), cohort 2 (B) and cohort 3 (C).

changes to several key regulators involved in metabolic function from small volumes of plasma. Blood glucose (mmol L^{-1}) levels were also measured using an Accu-chek Active system (Roche Diagnostics, Castle Hill, NSW, Australia). Note that glucose was not assessed in the nonfasted condition following abstinence.

Glucose tolerance test (GTT)

To examine the effects of adolescent CIT exposure on glycaemic control, subsets of rats were subjected to a GTT following 6 h food deprivation. GTT (2 mg, i.p.) was conducted at the end of the exposure period (n = 8–10 per group, PND 56) and abstinence (WC + SC, n = 8–10 per group, PND 84) followed by an additional GTT at a higher concentration of glucose (20 mg, i.p.) (SC, n = 8–10 per group; WC + SC, n = 8–10 per group, PNDs 85–87, Figure 1A). Blood glucose was determined via the tail vein using an Accu-chek Active system (Roche Diagnostics) before and 15, 30, 60, 90 and 120 min following glucose challenge. Plasma insulin concentrations (ng·mL^{-1}) were determined from tail blood samples (~30 μL) collected before and 15 and 30 min following glucose, which covers the known period of maximal response following glucose ingestion. Samples were centrifuged (5000 *g*, Eppendorf 5415C) at 4°C, and the resulting plasma insulin levels were determined via ELISA (Briggs *et al.*, 2014).

Hypothalamic tissue collection

To assess the expression of genes encoding hypothalamic neuropeptides, hypothalami were collected 72 h following the exposure period (cohort 3, n = 6 per group, PND 55, Figure 1C) and at the end of abstinence in rats given access to SC (n = 5–6 per group) or WC + SC (n = 7–8 per group). After the rats had been killed (Lethabarb, 1 mL·kg^{-1}, i.p), brain and peripheral organs were removed and weighed. Brains were placed in an ice-cold rat brain block and a ~2 mm thick slice of hypothalamus, containing the PVN, ARC and surrounding lateral, anterior and posterior hypothalamus dissected (Ganella *et al.*, 2013) from Bregma −0.8 to −2.90 mm, snap frozen over liquid nitrogen and stored at −80°C. As described for blood samples, all tissue was collected 2–4 h into the light cycle in a randomized manner between air and CIT-exposed rats.

RNA extractions

Hypothalamic RNA extractions and qPCR analysis were conducted as previously described (Briggs *et al.*, 2010; 2011; Ganella *et al.*, 2013). RNA was extracted using the RNeasy Plus Mini kits with genomic DNA eliminator (Qiagen, Melbourne, Vic., Australia) as per the manufacturer's instructions. For RNA, 400 ng of RNA was reverse transcribed into cDNA using the Prime Script™ reverse transcription Kit (Takara Bio Inc., Otsu, Shiga, JP) with random hexamers and Oligo dT primers as per the manufacturer's instructions. Reverse transcription reactions were performed on a PCR Thermal Cycler Dice (Takara Bio Inc.) using the following conditions: 37°C for 15 min and 85 C for 5 s and then held at 4°C. The cDNA products were diluted 1:10 in nuclease-free water (Ambion, Foster City, CA, USA) and stored at −20°C.

qPCR

Relative gene expression was conducted using qPCR performed on a ViiA™ 7 Real-Time PCR system (Applied Biosystems, Waltham, MA, USA) using standard conditions: 95°C, 5 min; 40 cycles, 95°C for 10 s; and 60°C, 1 min. All qPCR experiments were conducted in triplicate in 10 μL reactions containing 2 μL cDNA, 5 μL SYBR Green Mastermix (Applied Biosystems), forward and reverse primers and nuclease-free water (Ambion). Determination of relative gene expression was conducted using the $2^{-\Delta\Delta CT}$ method (Livak

and Schmittgen, 2001; Schmittgen and Livak, 2008). Exon spanning primers were designed using primer 3 (Untergasser *et al.*, 2012), and *hypoxanthine phosphoribosyltransferase 1* (*Hprt1*) was employed as an endogenous control (Ganella *et al.*, 2013). Primer sequences for *Hprt1*, *agouti-related protein* (*Agrp*), *growth hormone secretagogue receptor/ghrelin receptor* (*Ghsr*), *insulin receptor* (*Insr*), *leptin receptor* (*Lepr*), *Npy*, *pro-opiomelanocortin* (*Pomc*) and *corticotropin-releasing hormone* (*Crh*) are indicated in Table 1. All primers were optimized and had primer efficiencies of 90–110%, and data are expressed as a fold change relative to air-exposed controls.

Data analysis and statistical procedures

Statistical analyses was conducted with SPSS 20 (IBM, Armonk, NY, USA) or SIGMASTAT 3.5 (SyStat Software Inc., San Jose, CA, USA), and graphs were produced with GRAPHPAD PRISM 6 (GraphPad Software, La Jolla, CA, USA). Body weights, food and water intake, glucose and insulin levels were analysed with two-way repeated measures (RM) ANOVA with time and treatment as factors and Tukey *post hoc* comparisons. Three-way RM ANOVAs were used to analyse food intake throughout abstinence (time, treatment and chow as factors). Quadratic regression analyses were performed to assess the relationship between food intake (kCal) and body weight. All body composition data, circulating hormone levels, body and organ weights and mRNA transcript expression analyses were analysed with unpaired *t*-tests. Statistical significance was taken at $P < 0.05$. Data are presented as mean ± SEM with the exception of the Echo-MRI data, which are presented as mean of means ± SEM.

Results

Effects of CIT on growth, food and water intake throughout the 4 week exposure period

Analysis of grouped body weights revealed a main effect of treatment [$F_{(1,\ 38)} = 14.60$, $P < 0.001$] and time [$F_{(2,\ 76)} = 5516.91$, $P < 0.001$] and significant treatment × time interaction [$F_{(2,\ 76)} = 51.47$, $P < 0.001$, Figure 2A]. While body weight increased for both groups during the exposure period, *post hoc* analysis revealed that body weights were significantly reduced in CIT-exposed rats compared with controls from the sixth exposure day ($P < 0.01$).

Analysis of food intake (g·day^{-1}) revealed a main effect of treatment [$F_{(1,\ 38)} = 24.27$, $P < 0.001$] and time [$F_{(2,\ 76)} = 593.67$, $P < 0.001$] and a significant treatment × time interaction [$F_{(2,\ 76)} = 26.82$, $P < 0.001$, Figure 2B]. *Post hoc* analysis revealed a significant decrease in food intake in CIT-exposed rats by the sixth exposure ($P < 0.01$), which remained decreased at the end of the exposure period ($P < 0.001$). To control for the effects of CIT on body weight, food intake was corrected for body weight. Analysis revealed a main effect of time [$F_{(2,\ 76)} = 61.83$, $P < 0.001$] and a treatment × time interaction [$F_{(2,76)} = 3.132$, $P = 0.049$, Figure 2C]. *Post hoc* analysis revealed no significant differences between groups. No significant differences in water intake were observed between treatment groups (Supporting Information S1).

The relationship between food intake and body weight was further investigated using quadratic regression. During the exposure period, body weight significantly predicted the daily kilocalorie intake for all animals [$F_{(1,\ 475)} = 269.27$, $P < 0.001$, adjusted $R^2 = 0.807$; Figure 2D], and CIT exposure significantly altered the relationship between body weight and kilocalorie intake ($P = 0.007$).

Effects of CIT exposure on body composition

Echo-MRI revealed no significant differences in fat, lean mass or total water content between air and CIT-exposed rats at baseline (PND 27, data not shown). On the final exposure day (PND 52), CIT-exposed rats displayed significantly less fat (air 9.65 ± 0.21%; CIT 8.68 ± 0.31%, $P = 0.014$), with no differences in lean mass (air 86.34 ± 0.20%; CIT 86.95 ± 0.37%) or total water (air 66.49 ± 0.30%; CIT 66.90 ± 0.61%) as percentages of total body weight.

Plasma glucose and metabolic hormone levels following exposure

Plasma levels of glucose and circulating metabolic hormones were assessed in both nonfasted and fasted conditions on PND 55 (Table 2). In the nonfasted condition, plasma levels of GLP-1 ($P = 0.025$) and IL-6 ($P = 0.031$) were significantly increased, while glucose ($P = 0.020$) and insulin ($P = 0.028$) were significantly decreased in CIT-exposed rats (Table 2). Following 12 h food deprivation, plasma levels of amylin ($P = 0.050$), glucose ($P = 0.024$), IL-6 ($P = 0.050$), MCP-1 ($P = 0.002$), PP ($P = 0.015$) and PYY ($P < 0.001$) were reduced in CIT-exposed rats compared with controls (Table 2).

Table 1

Primer sequences for qPCR

	Forward (5′-3′)	Reverse (5′-3′)
Hprt1	CTGGTGAAAAGGACCTCTCG	TCCACTTTCGCTGATGACAC
Agrp	GCTTTGGCAGAGGTGCTAGA	TTGAAGAAGCGGCAGTAGCA
Pomc	TCCTCAGAGAGCTGCCTTTC	ATGGAGGTCTGAAGCAGGAG
Npy	CCCGCCATGATGCTAGGTAA	CAGCCAGAATGCCCAAACAC
Ghsr	ACTCACTGCCTGACGAAC TG	GTGAGCAGGTTGCCTGAG
Lepr	CCAGTACCCAGAGCCAAAGT	GGGCTTCACAACAAGCATGG
Insr	GCCACAGTGTTGCTTGCAG	CATACTCCGCGAGAAGGTCG
Crh	AAATGGCCAGGGCAGAGCAGT	TGGCCAAGCGCAACATTTCAT

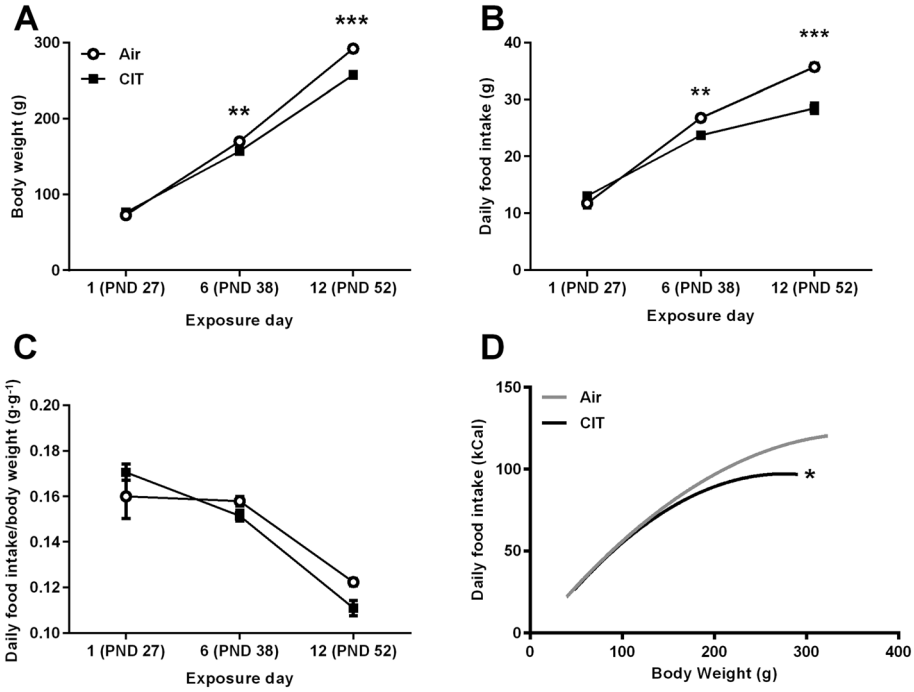

Figure 2

Body weight and food intake throughout the exposure period. Body weight (A) and food intake (B) of CIT compared with air-exposed rats were significantly reduced by the sixth exposure day (PND 38) with both decreased weights and food intake remaining at the end of the exposure period (PND 52, $^{**}P < 0.01$, $^{***}P < 0.001$, air vs. CIT, two-way RM ANOVA with Tukey *post hoc* comparisons). (C) When daily food intake was corrected for body weight, no significant differences were observed between groups (two-way RM ANOVA). (D) Quadratic regression analysis of daily kilocalorie intake versus body weights for air and CIT-exposed rats throughout the exposure period. Adolescent CIT exposure significantly altered the relationship between daily kilocalorie intake and body weight, as CIT-exposed rats consumed fewer kilocalories than air-exposed rats at the same body weight ($^{*}P < 0.05$).

Table 2

Plasma blood glucose and circulating hormones 72 h following the exposure period

	Non-fasted		Fasted	
Marker	Air	CIT	Air	CIT
Amylin (pg·mL^{-1})	62.4 ± 5.0	61.2 ± 5.5	69.3 ± 7.8	49.4 ± 5.2*
C-peptide (pg·mL^{-1})	2205.9 ± 87.4	2068.8 ± 93.3	1073.8 ± 102.6	878.4 ± 155.8
GIP (pg·mL^{-1})	177.9 ± 17.3	129.9 ± 16.6	25.7 ± 5.7	20.7 ± 3.1
GLP-1 (pg·mL^{-1})	109.4 ± 6.3	147.4 ± 14.2*	84.7 ± 11.5	64.4 ± 9.4
Glucagon (pg·mL^{-1})	52.7 ± 2.7	64.5 ± 3.6	45.8 ± 2.4	33.0 ± 4.6
Glucose (mmol·L^{-1})	6.7 ± 0.1	6.4 ± 0.1*	5.4 ± 0.18	4.9 ± 0.2*
IL-6 (pg·mL^{-1})	166.3 ± 26.8	355.8 ± 76.6*	322.7 ± 58.3	179.2 ± 37.6*
Insulin (pg·mL^{-1})	1761.1 ± 105.4	1349.6 ± 136.1*	573.0 ± 100.0	529.2 ± 103.2
Leptin (pg·mL^{-1})	8341.9 ± 1846.3	6374.3 ± 696.5	1949.1 ± 271.6	1609.5 ± 320.0
MCP-1 (pg·mL^{-1})	355.7 ± 31.2	436.2 ± 31.7	489.1 ± 34.1	343.3 ± 22.3*
PP (pg·mL^{-1})	62.3 ± 10.3	90.4 ± 15.7	83.2 ± 17.8	32.9 ± 4.5*
PYY (pg·mL^{-1})	180.3 ± 14.2	169.2 ± 17.1	154.3 ± 14.7	71.3 ± 11.5*

Data are presented as mean ± SEM, $n = 8$–10 per group.
$^{*}P < 0.05$, unpaired *t*-test compared with air controls.

GTT following exposure

Analysis of blood glucose levels following a GTT revealed a main effect of time only [$F_{(5,\ 90)} = 15.80$, $P < 0.001$], despite an evident reduction in blood glucose at 30 min in CIT-exposed rats compared with controls (Figure 3A). Plasma insulin levels following GTT revealed a main effect of treatment [$F_{(1,\ 15)} = 6.59$, $P = 0.021$], evident as a decrease in CIT compared with air-exposed controls (Figure 3B).

Body weight, composition and food intake: SC only during abstinence

Analysis of body weights throughout abstinence for SC animals revealed a main effect of treatment [$F_{(1,\ 18)} = 9.77$, $P = 0.006$] and time [$F_{(2,\ 36)} = 1032.11$, $P < 0.001$] yet no interactions (Figure 4A). This was evident as a sustained lower body weight of CIT compared with air-exposed rats. Echo-MRI following ~4 weeks of abstinence (PND 86) and access to SC revealed no significant differences in fat (air 12.53 ± 0.58%; CIT 12.64 ± 1.00%), lean mass (air 85.45 ± 0.89%; CIT 86.31 ± 0.98%) or total water content (air 64.83 ± 0.64%; CIT 64.82 ± 0.74%), suggesting normalization of body composition following abstinence from adolescent CIT exposure.

Analysis of food intake revealed a main effect of time [$F_{(2,\ 36)} = 8.31$, $P = 0.001$] and a time × treatment interaction [$F_{(2,\ 36)} = 4.62$, $P = 0.016$]. However, *post hoc* analysis revealed no significant differences (Figure 4C). When corrected for body weight, food intake throughout abstinence was increased in CIT compared with air-exposed rats [main effect of treatment, $F_{(1,\ 18)} = 4.53$, $P = 0.047$, and time, $F_{(2,\ 36)} = 100.54$, $P < 0.001$, Figure 4E]. There were no significant differences in water intake between treatment groups (Supporting Information S1).

Body weight, composition and food intake: western and standard chow during abstinence

In animals given access to both WC and SC, CIT animals had significantly lower body weight compared with controls throughout the abstinence period [main effect of treatment, $F_{(1,\ 18)} = 10.58$, $P = 0.004$, and time, $F_{(2,\ 36)} = 1332.86$, $P < 0.001$, Figure 4B]. At PND 86, Echo-MRI revealed no difference in body fat (air 20.31 ± 0.80%; CIT 19.40 ± 1.21%), lean mass (air 77.51 ± 1.17%; CIT 77.98 ± 1.18%) or total water content (air 58.77 ± 0.82%; CIT 59.07 ± 0.99%) as percentages of total body weight.

Analysis of total food intake (WC + SC) throughout abstinence revealed a main effect of treatment [$F_{(1,\ 18)} = 4.53$, $P = 0.047$] and time [$F_{(2,\ 36)} = 8.44$, $P = 0.001$], with decreased food intake in CIT compared with air-exposed rats (Figure 4D). When corrected for body weight, analysis of total food intake (WC + SC) revealed a main effect of time only [$F_{(2,\ 36)} = 207.95$, $P < 0.001$, full analysis in Supporting Information S1]. During abstinence, body weight significantly predicted daily kilocalorie intake for all animals [$F_{(1,\ 399)} = 6.7$, $P = 0.01$, adjusted $R^2 = 0.116$], and CIT exposure significantly altered the relationship between body weight and kilocalorie intake ($P = 0.001$) irrespective of diet.

For WC + SC rats, analysis of dietary preference (WC/SC) in weeks 1 and 4 of abstinence revealed a main effect of time [$F_{(1,\ 18)} = 5.69$, $P = 0.028$] and a treatment × time interaction [$F_{(1,\ 18)} = 22.15$, $P < 0.001$, Figure 4F]. *Post hoc* analysis revealed that during abstinence, air-exposed rats displayed a significant decrease in preference for WC from 1 to 4 weeks ($P < 0.001$, Figure 4F), which was not evident for CIT-exposed rats. Water intake was not significantly different between groups (Supporting Information S1).

Plasma metabolic hormone levels, response to GTT and saccharin preference: SC during abstinence

At the end of the abstinence period in both the nonfasted and fasted conditions, no significant differences were observed for any hormones assessed (Table 3), nor were differences in blood glucose or plasma insulin observed between groups following 20 mg glucose (i.p.) (Supporting Information S1). Notably, there was, however, a significant increase in saccharin preference in CIT compared with air-exposed rats (air 79.84 ± 5.81%; CIT 94.95 ± 1.05%, $P = 0.020$) at PND 96, indicating an augmented preference for sweet/palatable substances in rats with prior exposure to adolescent CIT.

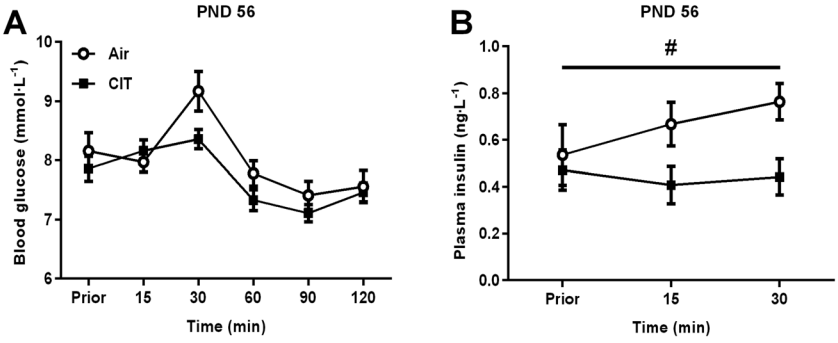

Figure 3

Blood glucose response to GTT following exposure. (A) Glucose challenge (2 mg, i.p.) in the GTT 4 days following the final exposure (PND 56). (B) Plasma insulin levels were significantly decreased in CIT-exposed rats following glucose challenge in the GTT ($^{\#}P < 0.05$, main effect of treatment, two-way RM ANOVA). Data are mean ± SEM, $n = 8$–10 per group.

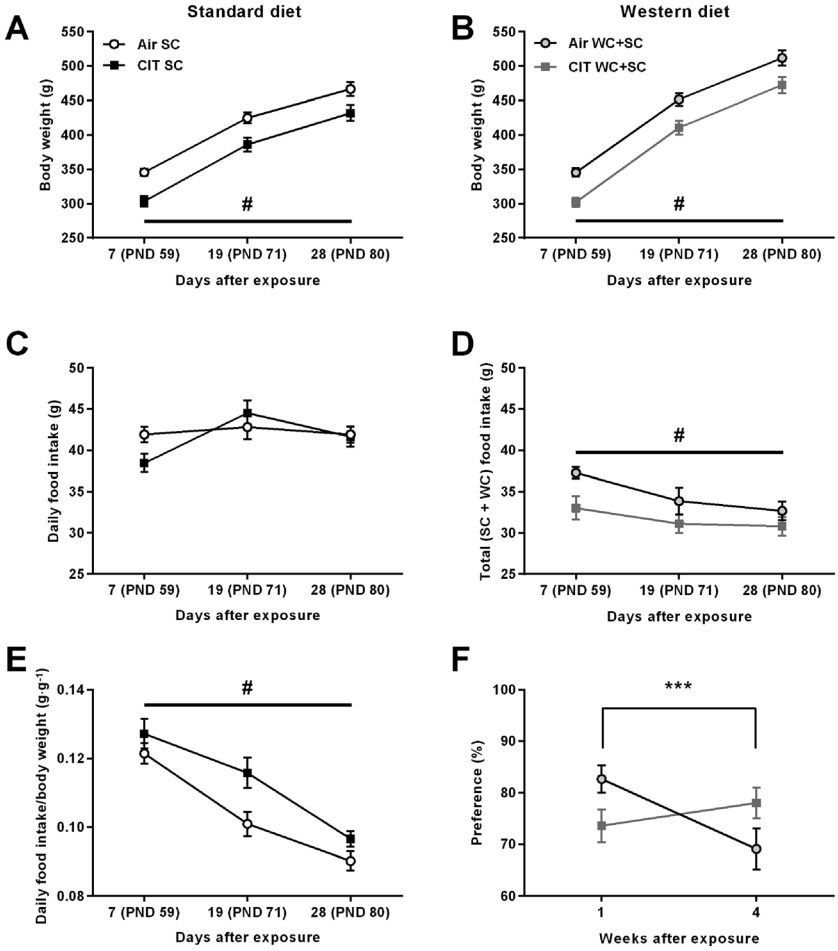

Figure 4

Body weight and food intake throughout abstinence following access to standard or western diet. Mean body weights throughout abstinence (PNDs 59–80) were significantly reduced in rats exposed to CIT compared with air-exposed rats when consuming either (A) SC or (B) WC + SC throughout abstinence ($^{\#}P < 0.01$ main effects of treatment, two-way RM ANOVA). (C) For rats consuming SC, average daily food intake was similar between treatment groups, yet when corrected for body weight (E), average food intake was significantly increased in CIT-exposed rats ($^{\#}P < 0.05$ main effect of treatment, two-way RM ANOVA). (D) For rats consuming WC, total food intake (WC + SC) throughout abstinence was significantly reduced in CIT compared with air-exposed rats ($^{\#}P < 0.05$ main effect of treatment, two-way RM ANOVA). (F) Air-exposed rats significantly decreased dietary preference for WC throughout abstinence, which was not evident in CIT-exposed rats (***$P < 0.001$, air week 1 vs. 4, two-way RM ANOVA with Tukey *post hoc* comparisons). Data are mean ± SEM, $n = 10$ per group.

Plasma glucose and metabolic hormone levels: western and standard chow during abstinence

In the nonfasted condition (PND 90), prior adolescent CIT exposure resulted in increased glucagon ($P = 0.033$) and decreased insulin ($P = 0.024$) levels compared with controls; no other significant differences were observed (Table 3). Following 12 h food deprivation (PND 83), CIT-exposed rats displayed increased GLP-1 ($P = 0.046$), leptin ($P = 0.006$) and MCP-1 levels ($P = 0.007$, Table 4).

GTT and insulin levels upon glucose challenge: western and standard chow during abstinence

Following a GTT (2 mg, i.p.) 4 weeks after the last exposure, there was a main effect of time [$F_{(5,\ 90)} = 33.62$, $P < 0.001$] and a time × treatment interaction [$F_{(5,\ 90)} = 3.81$, $P = 0.004$, Figure 5A] on blood glucose levels. *Post hoc* analysis revealed a significant increase of blood glucose levels in CIT-exposed rats at 30 min ($P = 0.022$) and a decrease at 120 min ($P = 0.022$) following GTT compared with air-exposed controls (Figure 5A). Plasma insulin levels following GTT revealed no main effect (Figure 5B). Similar responses were observed following 20 mg of glucose (i.p) (Supporting Information S1).

Body and organ weights: end of exposure and abstinence

In rats where tissues were collected at the end of the exposure period (PND 55), body weight was significantly reduced in CIT-exposed rats compared with controls by the sixth exposure day ($P < 0.01$, data not shown). Adolescent CIT exposure

Table 3

Plasma blood glucose and circulating hormones following access to standard chow throughout abstinence

	Non-fasted		Fasted	
Marker	Air	CIT	Air	CIT
Amylin (pg·mL^{-1})	125.2 ± 13.3	127.3 ± 11.6	53.1 ± 5.7	47.1 ± 4.6
C-peptide (pg·mL^{-1})	2462.3 ± 255.9	2378.7 ± 184.3	1266.7 ± 66.7	1323.8 ± 92.9
GIP (pg·mL^{-1})	139.5 ± 41.3	121.4 ± 23.9	22.5 ± 4.9	15.0 ± 2.8
GLP-1 (pg·mL^{-1})	86.1 ± 15.3	101.0 ± 5.6	63.7 ± 7.8	103.6 ± 21.7
Glucagon (pg·mL^{-1})	60.1 ± 6.7	58.3 ± 8.4	30.3 ± 3.4	33.3 ± 3.9
Glucose (mmol·L^{-1})	–	–	5.5 ± 0.2	5.3 ± 0.2
IL-6 (pg·mL^{-1})	163.7 ± 16.3	161.4 ± 21.8	185.5 ± 34.8	137.4 ± 17.0
Insulin (pg·mL^{-1})	2366.9 ± 320.6	2567.9 ± 297.5	768.5 ± 92.4	625.2 ± 8.9
Leptin (pg·mL^{-1})	32938.0 ± 6873.8	26999.0 ± 6174.4	8568.7 ± 1470.8	17288.0 ± 7171.6
MCP-1 (pg·mL^{-1})	332.6 ± 51.5	317.0 ± 16.0	404.7 ± 55.8	337.0 ± 28.2
PP (pg·mL^{-1})	82.9 ± 10.4	93.4 ± 17.7	62.8 ± 16.6	45.9 ± 6.1
PYY (pg·mL^{-1})	134.0 ± 17.7	138.0 ± 18.8	118.3 ± 10.5	90.4 ± 8.7

Data are presented as mean ± SEM, $n = 6$–10 per group.

Table 4

Plasma blood glucose and circulating hormones following access to western chow throughout abstinence

	Non-fasted		Fasted	
Marker	Air	CIT	Air	CIT
Amylin (pg·mL^{-1})	140.2 ± 8.5	120.0 ± 15.4	45.9 ± 3.0	50.4 ± 3.0
C-peptide (pg·mL^{-1})	3231.8 ± 229.7	2940.0 ± 600.2	2125.2 ± 202.5	2366.4 ± 234.9
GIP (pg·mL^{-1})	204.6 ± 47.7	357.8 ± 82.7	33.4 ± 7.5	38.4 ± 5.7
GLP-1 (pg·mL^{-1})	134.0 ± 12.0	128.4 ± 11.6	87.8 ± 7.4	108.3 ± 6.0*
Glucagon (pg·mL^{-1})	49.2 ± 4.2	76.1 ± 11.9*	28.0 ± 2.3	32.8 ± 1.7
Glucose (mmol·L^{-1})	–	–	6.1 ± 0.2	6.0 ± 0.1
IL-6 (pg·mL^{-1})	273.0 ± 69.2	268.6 ± 37.9	161.3 ± 32.4	205.2 ± 37.7
Insulin (pg·mL^{-1})	3404.3 ± 284.9	2211.6 ± 394.1*	1195.5 ± 150.7	1264.7 ± 147.1
Leptin (pg·mL^{-1})	34034.7 ± 6583.2	34043.0 ± 7430.7	13660.1 ± 2330.4	35807.7 ± 6552.2**
MCP-1 (pg·mL^{-1})	316.0 ± 19.5	366.3 ± 43.0	368.2 ± 20.2	450.0 ± 15.8**
PP (pg·mL^{-1})	50.2 ± 10.0	59.6 ± 15.2	36.9 ± 5.5	55.8 ± 7.3
PYY (pg·mL^{-1})	78.6 ± 11.6	78.4 ± 18.6	79.1 ± 11.0	131.7 ± 30.0

Data are presented as mean ± SEM, $n = 7$–10 per group.
*$P < 0.05$,
**$P < 0.01$, unpaired t-test compared with air controls.

increased brain ($P < 0.01$) and decreased liver ($P < 0.05$) weights 72 h following the last exposure. At the end of abstinence (PND 90), rats consuming SC had similar body and organ weights, irrespective of treatment. In rats given access to WC + SC, there was a significant reduction in body weight ($P < 0.05$) and increase in brain ($P < 0.01$) and heart weights ($P < 0.05$) in CIT compared with air-exposed rats (Supporting Table S1).

Hypothalamic mRNA expression

At the end of the exposure period (PND 55), hypothalamic mRNA transcript expression for *Lepr* in CIT-exposed rats was significantly reduced ($P = 0.029$, Figure 6A) with no significant differences in other transcripts assessed. Following consumption of SC throughout abstinence, rats previously exposed to adolescent CIT had a significant reduction in *Npy* ($P = 0.029$) and increased *Crh* ($P = 0.040$, Figure 6B) mRNA

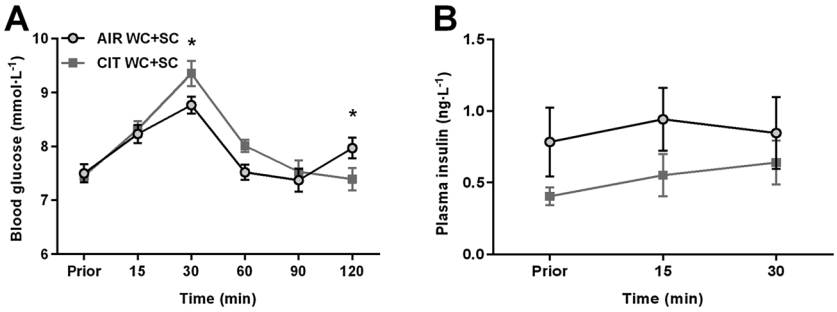

Figure 5

Blood glucose response to the GTT following access to WC. (A) For rats consuming WC + SC, glucose challenge (2 mg, i.p.) in the GTT at the end of the abstinence period revealed a significant difference in the blood glucose response between CIT and air-exposed rats (*$P < 0.05$, air vs. CIT, two-way RM ANOVA with Tukey *post hoc* comparisons). (B) No significant differences in plasma insulin levels were evident following glucose challenge in the GTT. Data are mean ± SEM, $n = 8$–10 per group.

expression, with no significant differences observed for other transcripts assessed. Following access to WC + SC during abstinence, there no were significant differences between groups despite an apparent increase in *Crh* mRNA expression in CIT compared with air-exposed rats (Figure 6C).

Discussion

We explored the relationship between adolescent inhalant abuse and metabolic dysfunction, especially parameters related to glycaemic control. We exposed rats to CIT at a high concentration (10 000 ppm) abused by humans, at a time point equivalent to early adolescence to reflect the age at which many young people experiment with inhalants (Lubman *et al.*, 2008; Duncan *et al.*, 2012). Exposure to CIT significantly affected food consumption and body weight, which was maintained even after sustained abstinence or when given access to a western diet. CIT also altered circulating levels of metabolic hormones and responses to a GTT suggesting alterations to factors mediating energy regulation and glycaemic control. In the brain, CIT during adolescence resulted in altered hypothalamic mRNA expression of genes involved in appetite and satiety implying a centrally mediated effect of CIT-induced alterations on energy homeostasis. Access to a western diet altered this relationship and perpetuated the inhalant-induced metabolic dysfunction observed at the end of the exposure period resulting in long-term changes in circulating metabolic hormones and glycaemic control even after sustained abstinence. Having established metabolic dysfunction following adolescent CIT exposure at high concentrations, our model can be further employed to investigate the effects of different CIT exposure paradigms upon metabolic parameters.

CIT exposure attenuated the normal gain in body weight after the sixth exposure, which was maintained even after abstinence. This concurs with observations in human adolescent abusers (Glaser and Massengale, 1962; Ryu *et al.*, 1998) and rodent models (Tyl *et al.*, 1999; Jarosz *et al.*, 2008; Soberanes-Chavez *et al.*, 2013; Dick *et al.*, 2014). CIT-induced changes in body weight coincided with decreased food consumption, and at the same body weight, animals exposed to CIT had reduced caloric intake, further supporting that toluene exposure results in changes to underlying processes mediating energy balance. Indeed, in the presence of volatile solvents, cells can regulate substrate switching between glucose and lipid metabolism (del Castillo and Ramos, 2007), which may explain reduced fat deposition in CIT-exposed rats observed at the end of the exposure period. Moreover, *Npy* mRNA expression after CIT exposure suggests a CNS-mediated hypermetabolic state, which may also aid in the explanation of decreased fat levels (see later in the text).

Metabolic hormones

In the nonfasted state, adolescent CIT exposure increased circulating levels of GLP-1 and IL-6 and decreased glucose and insulin levels, whereas in the fasted state, CIT exposure resulted in decreased levels of amylin, glucose, IL-6, MCP-1, PP and PYY when compared with air controls. Thus, although this study did not aim to directly compare results between fasted and nonfasted states, we did observe differential responses of metabolic factors dependent upon fasted state, as has been reported for other conditions in other studies (Frohli and Blum, 1988; Poirier *et al.*, 2001). While in simplistic terms decreased amylin and PYY levels, for example, should increase appetite (Huda *et al.*, 2006), we did not observe a corresponding increase in food intake in CIT rats. Furthermore, while changes to IL-6 may suggest that toluene has the ability to affect inflammatory responses, IL-6 levels in humans also correlate to glucose metabolism and insulin sensitivity (Heliovaara *et al.*, 2005). While full interpretation of these changes is limited due to the complex and overlapping signalling pathways involved, they do nevertheless provide evidence of CIT-induced metabolic dysfunction in this context. It must be noted that although samples were collected within a set time frame in this study, many metabolic parameters are subject to circadian regulation in rodents (Turek *et al.*, 2005) such that further investigation of metabolic parameters over a time course will be of interest to assess whether air and CIT-exposed rats display similar diurnal metabolic regulation.

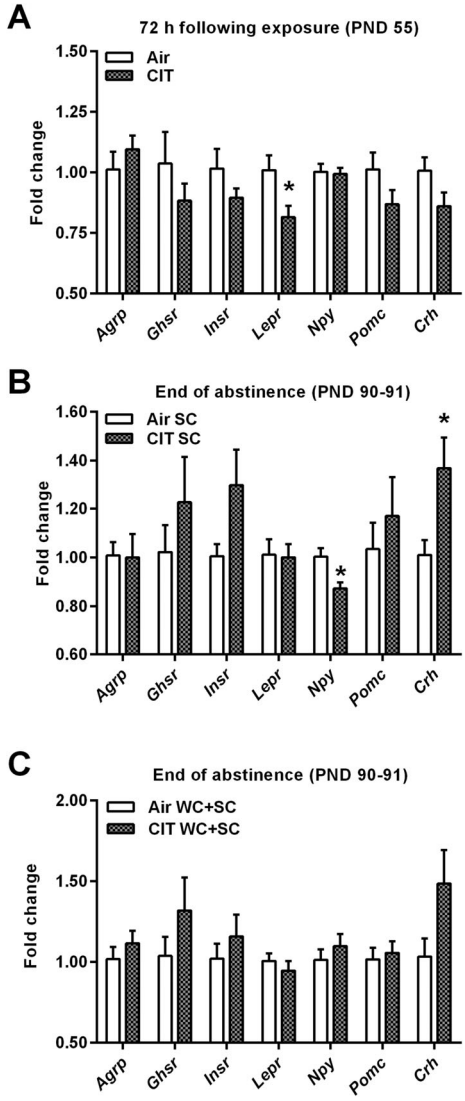

Figure 6

Effect of adolescent CIT exposure on hypothalamic levels of neuropeptide encoding genes. (A) Adolescent CIT exposure resulted in a significant reduction of hypothalamic *Lepr* mRNA expression 72 h (PND 55) following the final exposure ($n = 6$ per group). (B) Following access to SC throughout abstinence ($n = 5$–6 per group), prior exposure to adolescent CIT significantly decreased *Npy* and increased *Crh* transcript expression within the hypothalamus. Note that despite an apparent increase in *Insr* mRNA expression in CIT-exposed rats, this did not reach statistical significance ($P = 0.113$). (C) No significant differences in mRNA expression were observed between groups following access to WC + SC throughout abstinence ($n = 7$–8 per group). Note that despite an apparent increase in *Crh* mRNA expression in CIT-exposed rats, this did not reach statistical significance ($P = 0.089$). *$P < 0.05$, air versus CIT, unpaired *t*-test. Data are presented as mean ± SEM.

Amylin is also involved in glycaemic control via the regulation of gastric emptying whereas GLP-1, for example, is involved in inducing glucose-dependent stimulation of insulin secretion. Depleted insulin levels inhibit the ability of cells to take up glucose, and thus, cells switch to using alternative sources of energy, such as fatty acids. In humans, male industrial workers chronically exposed to low levels of volatile solvents including toluene also display altered fasting glucose and insulin levels (Won *et al.*, 2011), with continued exposure leading to insulin resistance (Won *et al.*, 2011). In our study, when challenged with a GTT 4 days following the exposure period, CIT-exposed rats failed to produce normal responses. This suggests that CIT exposure at a high concentration during adolescence results in impaired glucose metabolism.

CNS

While the underlying mechanisms involved in CIT-induced alterations in energy homeostasis and glycaemic control are likely to involve changes to peripheral organ systems, we observed a specific decrease in hypothalamic *Lepr* mRNA expression at the end of the exposure period, despite no changes in blood leptin levels at this time point (irrespective of fasted state). Inadequate receptor levels may alter the ability of leptin to regulate hypothalamic neurons, including NPY and POMC neurons (Skibicka and Grill, 2009). Thus, one mechanism driving the effects of CIT on altered energy regulation is that toluene exposure disrupts CNS-mediated regulation of feeding and glycaemic control. In rats, binge ethanol intake results in systemic insulin resistance driven primarily by impaired hypothalamic mediated responses (Lindtner *et al.*, 2013). As ethanol and toluene have similar properties (Lubman *et al.*, 2008), the insulin resistance reported in individuals exposed to inhalants (Won *et al.*, 2011), and changes observed in glycaemic control in our study may also occur via similar mechanisms. However, following abstinence, *Lepr* mRNA levels apparently normalized, suggesting the possibility of recovery of at least some systems.

Abstinence

Despite the apparent normalization of plasma metabolic hormones and GTT responses following 4 weeks of abstinence in rats maintained on SC, CIT-exposed animals remained significantly lighter than controls. Furthermore, *Npy* mRNA levels were significantly reduced, and *Crh* mRNA levels were significantly elevated in CIT-exposed rats, suggesting ongoing effects of CIT even after exposure has ceased. Acute exposure to toluene in adult rats is sufficient to alter NPY immunostaining in the PVN and ARC of the hypothalamus corresponding to a reduction in feeding behaviour and weight gain in these animals (Moron *et al.*, 2004). Reduced NPY indicates impaired negative feedback of energy sensing as NPY promotes energy conservation by switching off energy expenditure (Ruan *et al.*, 2014; Yulyaningsih *et al.*, 2014). Thus, low NPY represents a hypermetabolic state in CIT-exposed rats. In line with this, food intake in CIT-exposed animals relative to body weight is higher to maintain a hypermetabolic state. Furthermore, as NPY inhibits brown adipose tissue and white adipose tissue browning (Yulyaningsih *et al.*, 2014), decreased NPY is likely to reflect an increase in catabolism, potentially contributing to reduced fat levels observed following CIT.

Increased *Crh* mRNA expression is also likely to affect feeding behaviour as CRH acts in opposition to the actions of NPYand is involved in the suppression of appetite. Relative

to the changes observed in consumption in these animals, these results suggest hypothalamic dysfunction. Furthermore, it is important to consider that both NPY and CRH are involved in the regulation of stress, anxiety and food intake during stressful periods (Forbes and Cox, 2014), suggesting that adolescent CIT exposure may also increase a phenotype susceptible to stress and anxiety. Why the affects of CIT appear specific to *Npy* and *Crh* remains to be elucidated, the results are nevertheless indicative of CITs ability to disrupt CNS processes even following a period of sustained abstinence. As our analysis included the entire hypothalamus, further studies dissecting out the involvement of specific nuclei in toluene-induced hypothalamic discord will be of interest. Moreover, further investigation is required to confirm the functional implications of altered hypothalamic transcript expression upon protein levels within these regions.

Access to western diet

As exposure to toluene increases caloric and carbohydrate consumption in the industrial setting (Wang *et al.*, 1998), combined with our observation of a preference for sweet substances (despite no caloric value) even after sustained abstinence, we took a cohort of rats and gave them free access to either SC or WC (which contains four times more fat and 10 times more sucrose than SC) plus SC following CIT. Consumption of a western diet could perpetuate altered glycaemic control, such that even though fat levels do not increase in CIT-exposed animals beyond control levels, the nutrients preferentially consumed drive further metabolic dysfunction. Although rats given access to WC + SC put on significantly more weight than those maintained on SC alone, CIT-exposed rats still displayed lower body weights compared with controls. Furthermore, exposure to CIT significantly altered dietary preference and was still a predictor of daily caloric intake, irrespective of diet.

The CIT-induced changes to glycaemic control were perpetuated when combined with a western diet during abstinence, not dissimilar to altered glycaemic control in humans exposed to toluene (Won *et al.*, 2011). For rats given access to WC, CIT-exposed rats displayed hyperglycaemic and hypoinsulinaemic responses to both a low (2 mg) and high (20 mg) glucose challenge. Access to WC also altered circulating metabolic hormone levels; in the nonfasted state, glucagon was increased, and insulin was reduced in CIT compared with air-exposed rats. This would be expected to increase circulating glucose levels; however, glucose levels were not different between groups.

In the fasted state, CIT increased GLP-1 (as observed at the end of the exposure period), leptin and MCP-1 levels. Augmented levels of the adipocyte hormone leptin, for example, which were not different at the end of the exposure period, should restrict food intake; however, intake did not differ between groups. High leptin levels also impair the metabolic actions of insulin, which was significantly decreased in CIT-exposed rats in the nonfasted state, presumably impacting on glucose uptake (Muller *et al.*, 1997). Despite an apparent increase in *Crh* expression (similar to that observed for SC rats), no statistically significant differences in hypothalamic mRNA expression were observed in CIT-exposed rats subsequently given access to WC + SC, which may be due (in part) to increased variability of transcript expression. Despite this, altered levels of circulating metabolic hormones imply persistent effects on metabolic signalling at this time point.

Conclusions

Our data suggest that, in rats, CIT during adolescence results in impaired energy metabolism and leads to a phenotype of preferring sweet and/or fatty foods, which are preferentially consumed. We observed long-term changes in mRNA expression within the CNS, glycaemic control and metabolic hormone concentrations after CIT ceased. While the mechanisms underpinning inhalant-induced metabolic dysfunction require further investigation, our data suggest that they are likely to involve both negative feedback within the CNS and systemic changes. Thus, adolescent inhalant abuse results in metabolic instability resulting in persistent metabolic dysfunction, especially when combined with an unhealthy western diet.

Acknowledgements

The authors would like to thank Dr Michael Mathai (Victoria University) for access to the Echo-MRI and Mr Martin Axelsson for assistance with the saccharin preference studies. This study was supported by the National Health and Medical Research Council of Australia of which A. J. L. is a principal research fellow (1020737), the Australian Research Council (DP 110100379) of which J. R. D. was a future fellow during the time of the study (100100235) and the Victorian Government's Operational Infrastructure Support Scheme. The funding source had no involvement in the design, analysis and decision to publish.

Author contributions

All authors have made substantial contributions that meet the stated requirements for authorship:

- substantial contributions to the conception or design of the work or the acquisition, analysis or interpretation of data for the work;
- drafting the work or revising it critically for important intellectual content;
- final approval of the version to be published; and
- agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Specific contributions of each author include the following:

A. L. W. D., A. S., A. Q. and J. R. D. performed the majority of the experiments, data analysis and interpretation.

A. L. W. D., Z. A., A. J. L. and J. R. D. made substantial contributions to the concept of the study and undertook the majority of the manuscript preparation.

Z. A., A. J. L. and J. R. D. provided intellectual input regarding study design and interpretation of results.

Conflict of interest

There are no conflicts of interest in this study.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

http://dx.doi.org/10.1111/bph.13284

Table S1 Body and organ weights 72 h following the final exposure and at the end of the abstinence period.