Differential effects of hypercaloric choice diets on insulin sensitivity in rats

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Abstract

We showed previously that rats on a free-choice high-fat, high-sugar (fcHFHS) diet become rapidly obese and develop glucose intolerance within a week. Interestingly, neither rats on a free-choice high-fat diet (fcHF), although equally obese and hyperphagic, nor rats on a free-choice high-sugar (fcHS) diet consuming more sugar water, develop glucose intolerance. Here, we investigate whether changes in insulin sensitivity contribute to the observed glucose intolerance and whether this is related to consumption of saturated fat and/or sugar water. Rats received either a fcHFHS, fcHF, fcHS or chow diet for one week. We performed a hyperinsulinemic–euglycemic clamp with stable isotope dilution to measure endogenous glucose production (EGP; hepatic insulin sensitivity) and glucose disappearance (Rd; peripheral insulin sensitivity). Rats on all free-choice diets were hyperphagic, but only fcHFHS-fed rats showed significantly increased adiposity. EGP suppression by hyperinsulinemia in fcHF-fed and fcHFHS-fed rats was significantly decreased compared with chow-fed rats. One week fcHFHS diet also significantly decreased Rd. Neither EGP suppression nor Rd was affected in fcHS-fed rats. Our results imply that, short-term fat feeding impaired hepatic insulin sensitivity, whereas short-term consumption of both saturated fat and sugar water impaired hepatic and peripheral insulin sensitivity. The latter likely contributed to glucose intolerance observed previously. In contrast, overconsumption of only sugar water affected insulin sensitivity slightly, but not significantly, in spite of similar adiposity as fcHF-fed rats and higher sugar intake compared with fcHFHS-fed rats. These data imply that the palatable component consumed plays a role in the development of site-specific insulin sensitivity.

Key Words
- free-choice diet
- obesity
- insulin sensitivity
- Wistar rats

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Introduction

The prevalence of type 2 diabetes mellitus (T2DM), associated with obesity, is taking epidemic proportions. The molecular drivers involved in the pathogenesis of lower insulin sensitivity in obesity include, but are not limited to, increased concentrations of fatty acids and their intermediates, inflammatory changes, adipose tissue dysfunction, oxidative and ER-stress, and mitochondrial dysfunction (Reaven 2002,

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Morton & Barrett 2006, Chawla *et al*. 2011). Besides these mechanisms that are mainly related to increased body fat mass, palatable nutrients, that induce obesity-like (saturated) fat and sugar themselves, contribute to insulin resistance (Storlien *et al*. 1987, 1988, Pagliassotti *et al*. 1994, 1996, Cruciani-Guglielmacci *et al*. 2005). The differential impact of dietary fat and sugar on glucose metabolism might provide part of the explanation why not all obese individuals develop insulin resistance and/or T2DM. To investigate the impact of palatable (i.e. fat and sugar) nutrients on insulin sensitivity, it is important to use an animal model that mimics human daily consumption, which is characterized by the availability of calorie-dense palatable food items, either in solid or liquid form, that do not always contain minerals and vitamins.

Many different high-energy diets have been used to study insulin sensitivity in rodents in the past (Buettner *et al*. 2007), but these diets consist of a pellet which holds all nutrients. Because human daily consumption does not consist of a single solid food item without choice, we developed an obesogenic free-choice high-energy diet in which rats are offered two palatable items either in solid (pure fat (9 kcal/g)) or liquid form (sugar water (1.2 kcal/mL)), in addition to the normal balanced rat chow (3.31 kcal/g) and water. We observed that rats offered this free-choice high-fat, high-sugar (fcHFHS) diet showed persistent hyperphagia, characterized by an increase in meal number due to sugar intake without any compensatory decreases in meal size (la Fleur *et al*. 2007, 2010, 2014).

This diet affects metabolism rapidly, rats exposed to this free-choice high-fat, high-sugar (fcHFHS) diet for one week, show reduced glucose tolerance. Interestingly, rats that consume a free-choice high-fat (fcHF) diet (access to a dish of pure fat in addition to chow and tap water *ad libitum*) accumulate fat mass and increase circulating free fatty acids (FFA) similar to rats on a fcHFHS diet, but do not develop glucose intolerance (la Fleur *et al*. 2011). In addition, rats on a free-choice high-sugar (fcHS) diet (access to a 30% sugar solution in addition to chow and tap water *ad libitum*) consume more sugar water than rats on a fcHFHS diet, but do not accumulate fat mass, increase circulating FFA or become glucose intolerant (la Fleur *et al*. 2011), suggesting that dietary composition in a hypercaloric setting is an independent determinant of glucose metabolism and body composition. The short-term fcHFHS diet-induced glucose intolerance is not accompanied by an altered insulin response to an i.v. glucose bolus. Therefore, the question arises whether the glucose intolerance observed in rats on a fcHFHS diet is explained by reduced insulin sensitivity.

Changes in insulin sensitivity after consumption of a high-fat (HF) or high-sugar (HS) diet have been studied extensively, but mainly after at least one month of either increased sugar or fat intake which results in body weight gain with secondary metabolic changes like insulin resistance (Storlien *et al*. 1988, Kraegen *et al*. 1991, Pagliassotti *et al*. 1994, Pagliassotti & Prach 1995, Santure *et al*. 2003, Samuel *et al*. 2004, Alves *et al*. 2011). The short-term effects of HF and/or HS diets on glucose metabolism, as reported in our study has been less explored. Three days of HF feeding or exposure to a cafeteria diet (HF, HS) resulted in reduced hepatic insulin sensitivity but did not affect peripheral insulin sensitivity (Kraegen *et al*. 1991, Davidson & Garvey 1993), whereas exposure to the cafeteria diet for 7 days also altered peripheral insulin sensitivity (Davidson & Garvey 1993). We are unaware of studies using short-term, HS diets to investigate its effects on insulin sensitivity.

To investigate whether the glucose intolerance induced by the fcHFHS diet is explained by insulin resistance, we subjected rats to the fcHFHS diet for one week and performed a hyperinsulinemic–euglycemic clamp combined with stable isotope dilution to assess endogenous glucose production (EGP) and glucose disappearance (Rd) as measures of hepatic and peripheral body insulin sensitivity, respectively. To determine the separate role of saturated fat and of sugar water on insulin sensitivity, we added fcHF diet and fcHS diet groups to the study.

Materials and methods

Animals

Male Wistar rats (250–280 g) (Harlan, Horst, the Netherlands) were housed in Plexiglas cages in groups of four to six per cage in a temperature (20 ± 2°C), humidity (60 ± 2%) and light-controlled room with a 12/12 h light–darkness schedule (lights on at 0700 h). All animals had access to standard laboratory chow (special diet service, England) and tap water *ad libitum*. Rats were adapted to handling in the period before surgery. After surgery, rats were individually housed in Plexiglas cages (25 × 25 × 35 cm). The experiments were approved by the Committee for Animal Experimentation of the Academic Medical Centre of Amsterdam, the Netherlands.

Surgery

Rats were anesthetized with an i.p. injection of 80 mg/kg Ketamin (Eurovet Animal Health, Bladel, the

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Netherlands), 8 mg/kg Rompun (Bayer Health Care) and 0.1 mg/kg Atropin (Pharmachemie B.V., Haarlem, the Netherlands), after which a silicone catheter was implanted in the right jugular vein (according to the method of Steffens (1969) and left carotid artery for i.v. infusions and blood sampling, respectively. Catheters were fixed on the skull with dental cement. Rats received a recovery period of 7 days, during which they were handled daily to minimize stress.

Diet

After a recovery period of 7 days, rats were switched to either (1) a fcHFHS diet ($n=10$), that is, access to a dish of saturated fat (beef tallow (Ossewit/Blanc de Boeuf), Vandermoortele, Belgium) and a bottle of 30% sugar water (1.0 M sucrose mixed from commercial-grade sugar and water) *ad libitum*, in addition to their standard pellet chow and water bottle; (2) a fcHF diet ($n=8$), that is, access to a dish of saturated fat (beef tallow (Ossewit/Blanc de Boeuf), Vandermoortele, Belgium) *ad libitum*, in addition to their standard pellet chow and water bottle; (3) a fcHS diet ($n=7$), that is, access to a bottle of 30% sugar water *ad libitum*, in addition to their standard pellet chow and water bottle; or (4) only standard pellet chow ($n=12$). Body weights were matched among groups at the start of the diet.

Hyperinsulinemic euglycemic clamp and stable isotope infusion

All rats were subjected to the hyperinsulinemic euglycemic clamp combined with stable isotope enrichment to assess EGP and rate of disappearance of glucose (Rd). The experiment was performed in the rat's home cage.

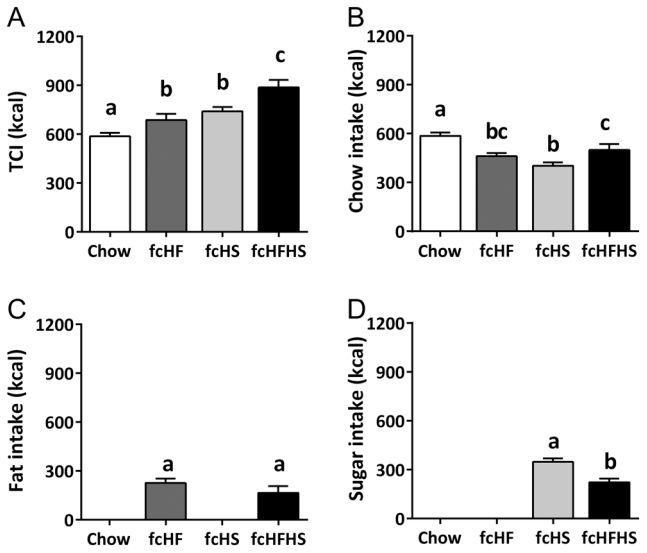

Figure 1
(A) Total caloric intake (TCI) after one week of rats on chow, fcHF, fcHS or fcHFHS diets. Rats on either choice diet showed increased TCI compared to chow-fed rats (fcHF vs chow $P=0.04$; fcHS vs chow $P=0.004$; fcHFHS vs chow $P<0.001$), and rats on fcHFHS diet had higher intake compared to rats on fcHS and on fcHF diet ($P=0.007$ and $P<0.001$ respectively). (B) fcHF-, fcHS- and fcHFHS-fed rats consumed less chow then chow-fed rats ($P=0.002$, $P<0.0001$ and $P=0.017$ respectively), and rats on fcHFHS diet consumed more chow than rats on fcHS diet ($P=0.019$). (C) Overall fat intake was not significantly different between fcHF- and fcHFHS-fed rats ($P=0.27$). (D) Overall intake of sugar solution was significantly higher in fcHS compared to fcHFHS-fed rats ($P=0.001$). Data are mean ± S.E.M. Different letters represent significant changes according to *post hoc* tests after ANOVA detected a significant effect of *diet* (for detail: Table 1). Two letters show that a group is significantly different compared to one group (with different letter) but not to another group (with similar letter).

Table 1 Statistics.

	One-way ANOVA's
Figure 1	
Total caloric intake	$F(3,36)=15.6$, $P<0.001$
Chow intake	$F(3,36)=8.7$, $P<0.001$
Figure 2	
Body weight	$F(3,36)=0.8$, $P=0.51$
WAT	$F(3,34)=5.7$, $P=0.003$
Basal plasma leptin	$F(3,35)=2.8$, $P=0.05$
Basal plasma FFA	$F(3,35)=2.5$, $P=0.07$
Table 2	
BW gain	$F(3,36)=1.3$, $P=0.30$
Basal blood glucose	$F(3,36)=0.8$, $P=0.49$
Basal plasma glucagon	$F(3,33)=0.4$, $P=0.77$
Basal plasma insulin	$F(3,36)=6.8$, $P=0.001$
Basal EGP	$F(3,36)=0.05$, $P=0.99$
Figure 3	
EGP suppression by insulin	$F(3,36)=3.6$, $P=0.02$
Rd	$F(3,36)=2.7$, $P=0.06$
Figure 4	
Clamp blood glucose	$F(3,36)=-1.1$, $P=0.35$
Clamp plasma insulin	$F(3,36)=1.4$, $P=0.25$
Clamp plasma glucagon	$F(3, 33)=0.4$, $P=0.73$
Clamp plasma FFA	$F(3,35)=2.9$, $P=0.05$
FFA suppression by insulin	$F(1,35)=1.7$, $P=0.19$
Figure 3	**rmANOVA**
GIR	
Time	$F(4,132)=8.632$, $P<0.0001$
Diet	$F(3,33)=3.54$, $P=0.0251$
Time * diet interaction	$F(12,132)=3.258$, $P=0.0004$
Tukey's multiple comparisons test	
Chow vs fcHF	$P=0.1122$
Chow vs fcHS	$P=0.4584$
Chow vs fcHFHS	$P=0.0219$
fcHF vs fcHS	$P=0.9004$
fcHF vs fcHFHS	$P=0.9542$
fcHS vs fcHFHS	$P=0.6172$

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To avoid interference of high amounts of circulating lipids, due to binge-like fat intake, or glucose derived from stomach content, we first determined whether after 4 h of fasting plasma was lipemic and/or there was food left in the stomach. Stomach content did not differ between animals in the different diet groups, but there was a clear lipid layer in plasma of many of the animals on the fcHF diet and even more for animals on the fcHFHS diet. As lipids infused directly in the blood stream can reduce insulin sensitivity (Lam *et al*. 2003, Tang *et al*. 2013, Pereira *et al*. 2014), we therefore choose to provide animals with chow only the evening before the clamp and remove the saturated fat and/or sucrose from the cage. All rats received 20 g of chow. On the day of performing the clamp, food was removed 4 h before the start of the clamp at lights on and animals were connected to the blood sampling and infusion catheters. The catheters were kept out of reach by means of a counterbalanced beam. This allowed all manipulations to be performed outside the cages without handling the animals.

Each clamp experiment consisted of a basal equilibration period (t=0–100) to measure basal EGP and a hyperinsulinemic, euglycemic clamp period (t=110–250) to assess hepatic and peripheral insulin sensitivity. At 1000 h (t=0), a primed (6 µmol in 5 min) followed by a continuous [6.6-$^2H_2$] glucose (>99% enriched; Cambridge Isotope Laboratories, Cambridge, MA, USA) (3000 µL/h) infusion was started using an infusion pump (Harvard Apparatus, Holliston, Massachusetts, USA). Five minutes before infusion (t=–5), a blood sample was drawn to measure background isotopic enrichment. After 90 min of equilibration time, three blood samples (200 µL) were drawn at t=90, 95, 100 to measure basal concentrations of blood glucose and basal plasma concentrations of insulin, glucagon, leptin and FFA and to determine isotopic enrichment during the equilibration state. Basal concentrations of blood glucose and plasma leptin, insulin, glucagon and FFA are calculated as the average concentration of these three blood samples. Following the last equilibration blood sample, insulin (Actrapid, Novo Nordisk) was administered in a primed 7.2 U/kg·min for 4 min) followed by a continuous intravenous infusion (3 U/kg·min). Euglycemia (5.5 ± 0.3 mmol/L) was maintained by a variable infusion of 25% glucose solution (enriched with 2.35% [6,6-D2] glucose) via the jugular vein catheter. Blood glucose concentrations were measured every 10 min from carotid artery samples and infusion rate of glucose was adjusted. At the end of the clamp, five blood samples were drawn with a 10 min interval from t=210 to t=250. From these blood samples we measured the following parameters: blood glucose concentrations, plasma concentrations of insulin, leptin, glucagon and FFA and isotopic enrichment. Concentrations of blood glucose and plasma leptin, insulin glucagon and FFA during the clamp are calculated as the average concentration of these five blood samples. After the clamp, all rats were killed by injection of Pentobarbital (~50 mg/mL; 0.3 mL) via the carotid artery catheter. Individual mesenteric (MWAT), perirenal (PWAT), epididymal (EWAT) and subcutaneous (SWAT) white adipose tissues (WAT) were dissected from the left side and weighed.

Analytical methods

Blood glucose concentrations were directly measured during the experiment, using a custom glucose meter (Freestyle Freedom Lite, Abbott). Blood samples were immediately chilled on ice in Eppendorf tubes with 5 µL heparin: saline (10×) solution and centrifuged at RT (15 min, 1600 ***g***). Plasma was stored at –20°C until further analysis. Plasma concentrations of insulin and glucagon were measured using radioimmunoassay kits (Millipore and Biochemicals, Costa Mesa, CA, respectively). The amounts of sample, standards, label, antibody and

Table 2 Basal concentrations of blood glucose, plasma glucagon, insulin and basal EGP in rats on a chow, fcHF, fcHS or fcHFHS diet.

	Chow		fcHF		fcHS		fcHFHS	
	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
BW gain (g)	40.3	3.4	39.1	3.8	37.6	1.7	47.1	4.3
Glucose (mmol/L)	5.67	0.10	5.74	0.12	5.55	0.14	5.48	0.16
Glucagon (ng/mL)	95.81	9.87	91.76	4.15	103.64	4.03	95.25	5.16
Insulin (ng/mL)	2.23	0.16	3.68	0.54	3.95*	0.52	4.52*	0.52
EGP (µmol/kg·min)	52.44	3.00	51.27	5.75	53.13	2.24	51.53	2.52

fcHF, free-choice high-fat; fcHS, free-choice high-sugar; fcHFHS, free-choice high-fat, high-sugar.
*P<0.03 vs chow.

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precipitating reagent, described in the manufactures' protocol, were divided by four.

Previous research from our department has shown that the mean cross-reactivity of Actrapid measured in rat plasma using the insulin radioimmunoassay is 84% (Ackermans, Ann Clin Biochem, 2008). Plasma [6,6-$^2H_2$] glucose enrichment was measured by gas chromatography–mass spectrometry (GCMS) (Ackermans *et al*. 2001), EGP and Rd were calculated using Steele equations (Steele 1959). The FFA concentration was determined with an enzymatic colorimetric method (NEFA-HR(2) test kit, Wako Chemicals GmbH).

Statistics

All data are presented as means ± S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA) (SPSS). Glucose infusion rate (GIR) was analyzed with repeated-measure analysis of variance (rmANOVA)

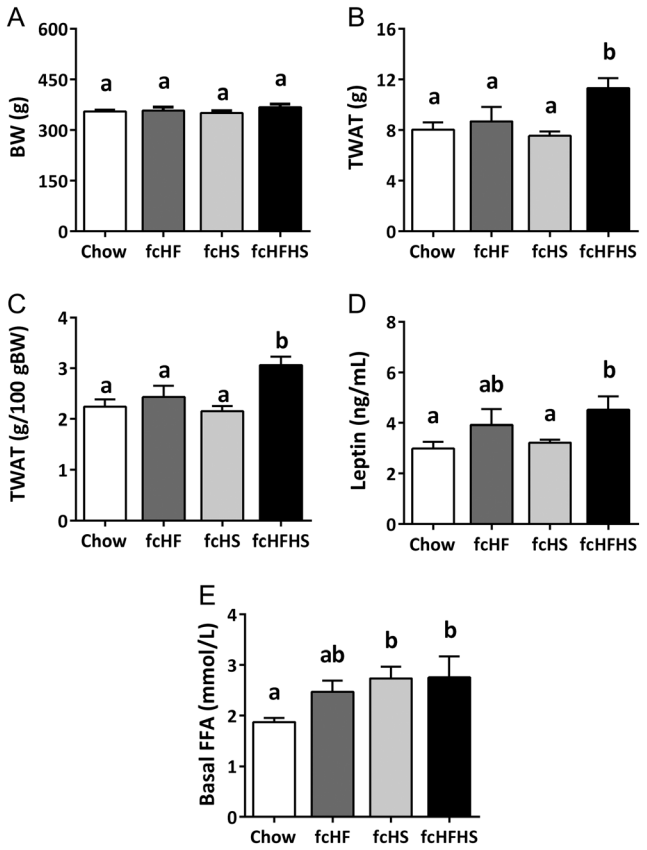

Figure 2
(A) Body weights were not different between choice diet groups after one week. (B) Total fat mass (sum of mesenteric, epidydimal, perirenal and subcutaneous) was significantly increased in fcHFHS rats and different from all other groups ($P<0.03$). Differences in fat mass between the groups was not because of one specific fat depot, all depots changed similarly. (C) Percentage total fat mass of the four individual fat pads per 100 g BW. (D) Plasma leptin concentrations were significantly higher in fcHFHS-fed rats compared to chow and fcHS-fed rats ($P=0.011$ and $P=0.05$ respectively). (E) Plasma FFAs tended to be different overall ($P=0.07$, Table 1). FFA was significantly higher in fcHS vs chow ($P=0.04$) and in fcHFHS vs chow ($P=0.02$). Data are mean ± S.E.M. Different letters represent a significant difference between groups, after ANOVA detected a significant effect of *diet* (details for statistics: Table 1). Two letters show that a group is significantly different compared to one group (with different letter) but not to another group (with similar letter).

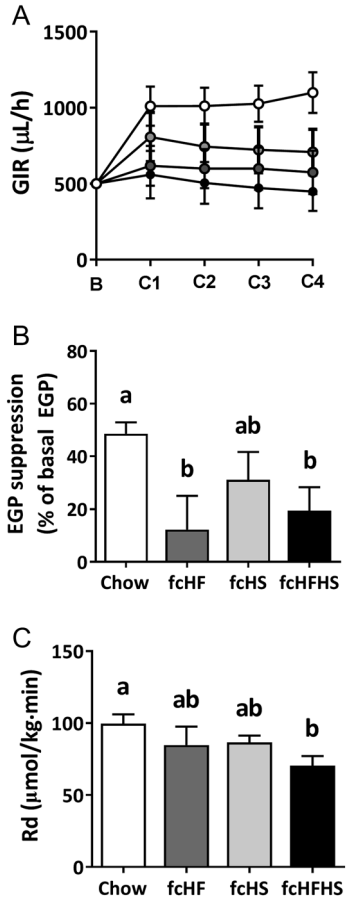

Figure 3
(A) Glucose infusion rate (GIR) during the hyperinsulinemic, euglycemic clamp in rats on a chow, fcHF, fcHS or fcHFHS diet for one week. B: basal, C1–4: intervals between the 5 blood samples drawn during the hyperinsulinemic, euglycemic clamp. GIR was significantly decreased in fcHFHS-fed rats compared to chow-fed rats (Table 1). (B) Suppression of endogenous glucose production (EGP) during the hyperinsulinemic euglycemic clamp in rats on a chow, fcHF, fcHS or fcHFHS diet for one week. EGP suppression was lower in fcHF-fed and in fcHFHS-fed rats compared to chow-fed rats ($P=0.005$ and $P=0.016$ resp.). (C) Peripheral glucose uptake (rate of disappearance, Rd) in rats on a chow, fcHF, fcHS or fcHFHS diet for one week was significantly lower in rats fed a fcHFHS diet compared to chow-fed rats ($P=0.008$). Data are mean ± S.E.M. Statistical differences are represented according to the *post hoc* with $P<0.02$, after ANOVA detected a significant effect of *diet* (Table 1). Different letters represent a significant difference between groups, after ANOVA detected a significant effect of *diet* (details for statistics: Table 1).

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(SPSS) to test for effects of *Time, Diet* and *Time*Diet* interaction. *Post hoc* LSD Fisher analysis was performed to detect individual group differences if ANOVA detected a significant effect.

Results

Adding access to saturated fat and/or sugar water to tap water and regular chow *ad libitum*, resulted in increased intake in fcHS-, fcHF- and fcHFHS-fed rats compared with chow-fed rats, with fcHFHS-fed rats consuming most calories (Fig. 1A, details on statistics given in Table 1). Analysis of different food components revealed that all free-choice diet rats reduced intake of chow compared with the chow-fed group, with the lowest intake observed in the fcHS group, which had significantly lower intake compared with the fcHFHS diet group, although similar compared with the fcHF diet group (Fig. 1B). Total fat intake over one week was not significantly different between fcHF- and fcHFHS-fed rats (Fig. 1C), whereas intake of the sugar solution was significantly higher in fcHS-fed compared with fcHFHS-fed rats (Fig. 1D).

One week of choice diets did not result in different body weight gain (Table 2), and thus the body weight on the day the hyperinsulinemic clamp was performed was not different between the groups (Fig. 2A). However, total WAT mass was different between the diet groups; rats on a fcHFHS diet had more WAT compared with chow-, fcHF- and fcHS-fed rats (Fig. 2B). Plasma concentrations of leptin were significantly higher in fcHFHS-fed rats compared with chow- and fcHS-fed rats, and similar to those fed the fcHF diet (Fig. 2C). Basal plasma FFA concentrations were significantly higher in rats on the fcHFHS and fcHS diet compared with chow-fed rats, but not significantly different from animals on the fcHF diet (Fig. 2D).

Before the hyperinsulinemic euglycemic clamp, animals were food deprived for 4 h at the beginning of the light period (i.e. during their inactive period). Basal concentrations of blood glucose, plasma glucagon and basal EGP were not different between rats on either diet (Table 2). However, basal plasma insulin concentrations in the fcHS and in the fcHFHS group were significantly higher compared with chow-fed rats (Table 2).

rmANOVA indicated an effect of *Time* and a *Time*Diet* interaction effect for GIR. Further analysis revealed that GIR was significantly decreased in fcHFHS-fed compared with chow-fed (Fig. 3A) rats. Further calculations and analysis of the data obtained from the stable isotope dilution revealed clear effect of consuming saturated fat in addition to chow on insulin-induced suppression of EGP as both rats on a fcHFHS and fcHF diet showed significant reductions in EGP suppression. No significant difference was detected between fcHS-fed and chow-fed rats (Fig. 3B). Furthermore, overall analysis revealed a strong trend ($P=0.06$) toward difference between diet groups for Rd, which was due to a significantly ($P=0.008$) lower Rd during hyperinsulinemia in the fcHFHS-fed compared with the chow-fed rats (Fig. 3C).

During hyperinsulinemia, concentrations of glucose, insulin and glucagon (Fig. 4A, B, C) were not different between groups, whereas concentrations of FFAs were significantly higher in fcHFHS-fed rats compared with chow- and fcHS-fed rats (Fig. 4D). Insulin-induced

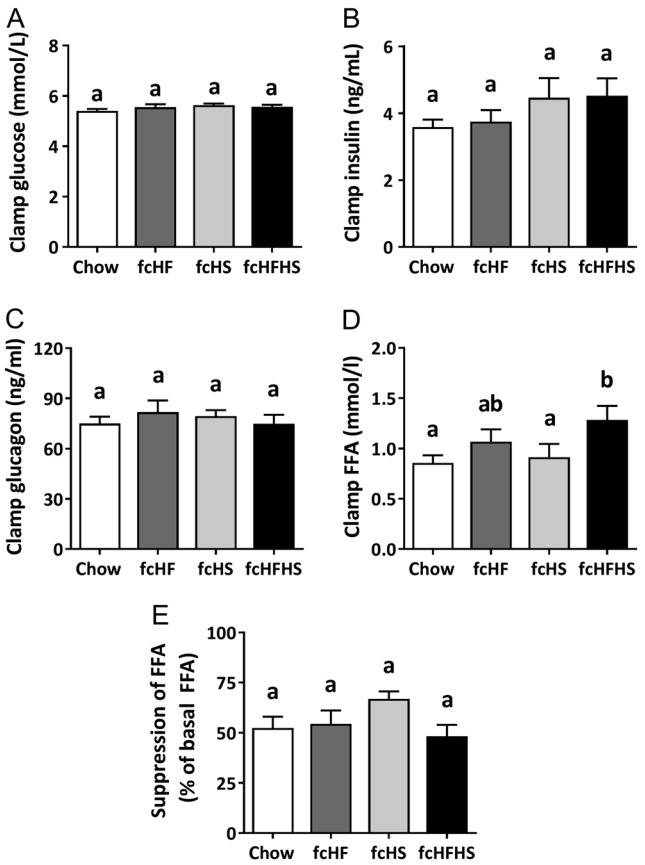

Figure 4
(A) Blood glucose concentrations and plasma concentrations of (B) insulin and (C) glucagon during the clamp were not significantly different, whereas (D) plasma concentrations of FFA were significantly higher in rats fed fcHFHS compared to chow-fed rats ($P=0.009$) and compared to fcHS-fed rats ($P=0.04$). (F) Suppression of FFA by insulin during the clamp was not different between the groups. Data are mean ± S.E.M. Details on statistics are depicted in Table 1. Two letters show that a group is significantly different compared to one group (with different letter) but not to another group (with similar letter).

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suppression of plasma FFA concentrations was not significantly different between diet groups (Fig. 4F).

Discussion

We here show that when rats consume excess saturated fat for a short period of time, either on a fcHF diet or on a fcHFHS diet, hepatic insulin sensitivity (i.e. lower insulin-induced EGP suppression) decreases significantly compared to the chow control group. In addition, when rats consume the combination of excess saturated fat and sugar (fcHFHS diet), their peripheral insulin sensitivity, mainly reflecting insulin action in skeletal muscle (Thiebaud *et al*. 1982), declines. Interestingly, although equally adipose and hyperphagic as rats on the fcHF diet, rats on the fcHS diet, which consumed only sugar water in addition to chow, did not show a significant decline in hepatic insulin sensitivity compared to the chow control group. These data suggest that the nature of the palatable component might play a role in site-specific development of insulin resistance, whereby it seems that intake of fat is more important as opposed to sugar in the induction of hepatic insulin resistance. Furthermore, the glucose intolerance reported previously in fcHFHS-fed rats (la Fleur *et al*. 2011) is most likely mediated, in part, by peripheral insulin resistance.

The important role of saturated fat intake in the development of hepatic insulin resistance is in line with earlier studies in which short-term, HF diets resulted in hepatic insulin resistance assessed by use of the hyperinsulinemic clamp, or by assessing the insulin-signaling cascade (Kraegen *et al*. 1991, Samuel *et al*. 2004). Increased circulating FFA have been postulated as an important mediator of hepatic insulin resistance (Lam *et al*. 2003, Pereira *et al*. 2014). We here show, however, that before the start of the clamp (baseline) plasma FFA concentrations were similar between the three choice diet groups, and FFA during the clamp were only significantly higher in rats on the fcHFHS diet and not in fcHF-fed rats. This points to additional mechanisms involved in the induction of hepatic insulin resistance linked to consuming saturated fat.

Only rats on the fcHFHS diet showed reduced whole body insulin sensitivity, which is in line with findings of others (Davidson & Garvey 1993). These authors used a short-term cafeteria diet consisting of HF, HS snack items (like cookies) and sweetened condensed milk, which resulted in reduced peripheral and hepatic insulin sensitivity as well as increased adiposity in rats.

Short-term (one week) feeding of fat (or sugar) alone did not affect Rd, which is in line with early studies showing that pelleted HF diets mainly affect hepatic insulin sensitivity and energy expenditure (Kraegen *et al*. 1985, Storlien *et al*. 1986), whereas peripheral insulin resistance only developed after 3 weeks of HF diet feeding (Kraegen *et al*. 1991, Alves *et al*. 2011). Exposure to sugar water alone did not significantly affect EGP suppression and did not contribute to additional EGP suppression in fcHFHS-fed rats. Little is known about short-term effects of sugar diets on hepatic and peripheral insulin sensitivity as most studies have been carried out for a prolonged period. For example, hyper- as well as isocaloric studies with increased or similar BWs, using 62% or 68% HS diets for 3, 5 or 8 weeks resulted in hepatic and peripheral insulin resistance (Pagliassotti *et al*. 1994, Pagliassotti & Prach 1995, Chicco *et al*. 2003, Santure *et al*. 2003). Hepatic insulin resistance was also observed following 16 weeks of consuming a low-sucrose diet (18% of total cal) (Pagliassotti & Prach 1995). Together this indicates that the time course and site-specific insulin resistance development depends on the quantity of sugar and the exposure time of a sugar diet.

In line with this, we observed basal hyperinsulinemia, concomitant with normal glucose tolerance and basal euglycemia in rats after 4 weeks on a fcHS diet (la Fleur *et al*. 2011).

Taken together, our data point to a role for the combined short-term consumption of saturated fat and sugar water in the development of peripheral insulin resistance. Moreover, the finding that fcHFHS-fed rats had significantly reduced Rd, only in comparison to the chow-fed group, further supports a role for the consumption of the combination of saturated fat and sugar water. It should be noted, however, that in contrast to earlier studies, in which we showed equal fat mass and caloric intake over the first week of exposure to a fcHF and a fcHFHS diet for one week (la Fleur *et al*. 2010, 2011), we here observed higher caloric intake and higher total fat mass after one week on the fcHFHS diet group compared to the fcHF and the fcHS diet. We therefore cannot exclude that the rapid development of peripheral insulin resistance in the animals on a fcHFHS diet was an indirect, that is, via increased adipose mass due to increased overall intake, and not a direct effect of the fat and sugar combination of the diet. However, the earlier shown impaired glucose tolerance in the fcHFHS-fed compared to the fcHF-fed rats despite similar

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adiposity and overall intake (la Fleur *et al*. 2011) suggests a direct effect of diet composition on insulin sensitivity independent of fat mass.

It must be noted that most HS diets used, have a solid formula, while we used sugar in water solution (to mimic sugar-sweetened beverages of a Western-style diet). Differences in sugar textures could render different responses. For example, mice fed a sugar solution showed increased expression of glucose transporters and cholecystokinin in ileum tissue compared to mice fed an equal amount of metabolizable energy via a solid sugar diet (Ritze *et al*. 2014). In addition, we showed previously that fcHFHS-fed rats consume their dietary sugar component also during the light period (la Fleur *et al*. 2014) and circadian disruption has been shown to induce insulin resistance (Shi *et al*. 2013). This indicates that sugar drinking might affect insulin sensitivity indirectly. In addition, the night before the experiment palatable food items were removed to avoid direct interference of the fat and/or sugar consumption on insulin sensitivity. Lipid infusions have direct effects on insulin sensitivity (Lam *et al*. 2003, Pereira *et al*. 2014) and timing of sugar consumption is very different between animals on a fcHFHS and on a fcHS diet (la Fleur *et al*. 2014). A further advantage of providing the animals with only a standardized amount of chow the night before the clamp is that all animals consumed a similar amount of calories.

In short, we here show that short-term (one week) feeding of saturated fat and sugar water, that is, the fcHFHS diet, impaired hepatic and peripheral insulin sensitivity, which likely contributed to the glucose intolerance observed previously (la Fleur *et al*. 2011). Interestingly, consumption of the saturated fat component significantly affected hepatic insulin resistance. In addition, consumption of solely the sugar water component affected insulin sensitivity slightly, but not significantly, although these rats were comparable to the fcHF-fed rats with regard to adiposity and had higher sugar intake compared with the fcHFHS-fed rats. These data imply that reductions in site-specific insulin sensitivity, after hypercaloric feeding, depends in part on the nature of the palatable component consumed independent of body weight gain. More research is needed to assess the underlying mechanisms in site-specific insulin insensitivity.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
C D and S I F designed the research. C D and L E performed the experiments. C D and S I F analyzed the data. C D, M J S and S I F wrote the manuscript. M A, E F, A K, M J S and S I F reviewed and edited the manuscript. E F, A K, M J S and S I F contributed to discussion. All authors approved the final version of the manuscript.

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