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Review Article

Associations between postprandial insulin and blood glucose responses, appetite sensations and energy intake in normal weight and overweight individuals: a meta-analysis of test meal studies

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It is unclear whether postprandial blood glucose or insulin exerts a regulatory function in short-term appetite regulation in humans. The aim of this study was to investigate, by use of meta-analysis, the role of blood glucose and insulin in short-term appetite sensation and energy intake (EI) in normal weight and overweight participants. Data from seven test meal studies were used, including 136 healthy participants (ALL) (92 normal weight (NW) and 44 overweight or obese (OW)). All meals were served as breakfasts after an overnight fast, and appetite sensations and blood samples were obtained frequently in the postprandial period. Finally, an *ad libitum* lunch was served. Data were analysed by fixed effects study level (SL) meta-regression analysis and individual participant data (IPD) regression analysis, using STATA software. In SL analysis, postprandial insulin response was associated with decreased hunger in ALL, NW and OW ($P<0{\cdot}019$), and with increased satiety in NW ($P=0{\cdot}004$) and lower subsequent EI in OW ($P=0{\cdot}022$). Multivariate IPD analysis showed similar associations, but only in NW for hunger, satiety and EI ($P<0{\cdot}028$), and in ALL for EI ($P=0{\cdot}016$). The only association involving blood glucose was the multivariate IPD analysis showing an inverse association between blood glucose and EI in ALL ($P=0{\cdot}032$). Our results suggest that insulin, but not glucose, is associated with short-term appetite regulation in healthy participants, but the relationship is disrupted in the overweight and obese. We conclude that the postprandial insulin response may be an important satiety signal, and that central nervous system insulin resistance in overweight might explain the blunted effect on appetite.

Insulin: Glucose: Short-term appetite regulation: Energy intake: Overweight

Fifty years ago, Mayer (1955) proposed that changes in blood glucose concentrations or arteriovenous glucose differences were registered by glucoreceptors situated in the parts of the hypothalamus that affect energy intake, and perhaps in the periphery as well. According to this theory, an increase in blood glucose concentrations results in increased feelings of satiety whereas a drop in blood glucose concentrations has the opposite effect. This glucostatic theory has received support from studies showing that transient declines in blood glucose concentrations preceded meal initiation (Campfield & Smith, 1990; Smith & Campfield, 1993; Anderson & Woodend, 2003). In addition, blood glucose concentrations following a meal have been shown to be inversely associated with subjective appetite and food intake (Anderson *et al.* 2002). However, another study found no associations between blood glucose concentrations and satiety, when blood glucose concentrations were raised by intravenous infusion (Lavin *et al.* 1996). Furthermore, several factors (e.g. insulin and incretin hormones) covary with postprandial blood glucose concentrations, which therefore makes it difficult to determine whether it is changes in blood glucose *per se* or other factors that are responsible for the satiety-promoting effect (Holt *et al.* 1996; Raben *et al.* 1996). Thus, it is not clear whether blood glucose *per se* is associated with short-term regulation of appetite.

Abbreviations: ALL, all participants; EI, energy intake; GLP-1, glucagon-like peptide 1; iAOC, incremental area over the curve; iAUC, incremental area under the curve; IPD, individual participant data; NW, normal weight; OW, overweight or obese; SL, study level; VAS, visual analogue scale.

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Like blood glucose, insulin has been suggested to be involved in short-term appetite regulation (Lavin *et al.* 1996). However, the results of insulin infusion studies have been conflicting since one of these studies has shown that increased insulin concentrations promote hunger and thereby increase energy intake at a subsequent *ad libitum* meal (Rodin *et al.* 1985), whereas others found no associations between insulin concentrations and appetite sensations (Woo *et al.* 1984; Gielkens *et al.* 1998). Furthermore, 7a recent study has shown that insulin concentrations before a test meal were inversely related to subsequent *ad libitum* energy intake (EI) in normal weight, but not in obese males (Speechly & Buffenstein, 2000). Thus, the role of insulin in short-term appetite regulation is controversial.

Since high concentrations of blood glucose and insulin are associated with obesity (Bonadonna *et al.* 1990), it seems possible that the potential effects of blood glucose and insulin in the short-term appetite regulation may differ between normal weight (NW) and overweight or obese (OW) participants.

Thus, the above-mentioned conflicting results call for more studies in order to clarify the roles of blood glucose and insulin in short-term appetite regulation. Therefore, the aim of the present study was to examine whether postprandial concentrations of blood glucose and insulin are associated with sensations of satiety and hunger, and subsequent *ad libitum* EI. Furthermore, we investigated whether these associations differ between NW and OW participants. A data set composed of participant data from six previously published studies and from one as yet unpublished study was used. To increase study power, these associations were analysed using meta-analysis.

Methods

Selection and inclusion of studies

We included all test meal studies that were conducted at the Department of Human Nutrition, the Royal Veterinary and Agricultural University, Denmark in the period 1990–2003, and fulfilled the criteria of inclusion of having individual participant data (IPD) on blood glucose, insulin and appetite scores (visual analogue scale (VAS)). From a total of 20 test meal studies, thirteen were excluded because they used *ad libitum* meals for breakfast, artificial meals, included only hyperinsulinaemic or diabetic participants, did not include appetite scores or because the data had not been analysed at the time of inclusion. Six of the seven studies that fulfilled all the above-mentioned criteria have been previously published (Flint *et al.* 1998, 2001, 2004*a*; Verdich *et al.* 2001*b*; Bakhøj *et al.* 2003; Raben *et al.* 2003), whereas the last study has been published as an abstract (Flint *et al.* 2004*b*).

Participants

From the seven studies, only the participants with complete data for blood glucose, insulin and appetite registrations were included in this meta-analysis. Eleven participants were excluded due to missing values, leaving 136 participants in the meta-analysis. Five of the participants were female. The sample size of the seven studies varied between ten and thirty-one. All of the participants were healthy, non-smoking non-athletes. The mean age of the participants was 29·4 years (SD 8·8). Ninety-two of the participants had a normal BMI ($\leq 25\,kg/m^2$) and were characterised as being NW, while the remaining 44 participants had a higher BMI ($>25\,kg/m^2$) and were characterised as being OW. All OW participants were male.

Design of the selected studies

In all studies, the participants were served a test meal in the morning after a 10–12 h fast. The test meal was followed by a postprandial period of 180–315 min (Table 1), during which insulin, blood glucose and sensations of hunger and satiety were measured frequently. In six of the studies, the participants were served an *ad libitum* meal at the end of the postprandial period. In the seventh study, the end of the postprandial period was the end of the study (Bakhøj *et al.* 2003). The macronutrient composition of the test meals varied markedly between the seven studies (for further information on meal type and composition, see the seven studies (Flint *et al.* 1998, 2001; 2004*a*,*b*; Verdich *et al.* 2001*b*; Bakhøj *et al.* 2003; Raben *et al.* 2003)).

In three of the studies, the effects of infused glucose-dependent insulinotropic polypeptide or glucagon-like peptide 1 (GLP-1) on different variables including appetite sensations and *ad libitum* EI were studied using a placebo-controlled randomised crossover design and blinding of participants (Flint *et al.* 1998, 2001) or participants and researchers (Flint *et al.* 2004*b*). From these studies, only data from the control situations were used. In three of the other studies, the effects of different test meals on different outcomes were examined using randomised crossover designs (Bakhøj *et al.* 2003; Raben *et al.* 2003; Flint *et al.* 2004*a*). In the

Table 1. Characteristics of the seven test meal studies

Author (year)	No. of participants (NW/OW)	BMI (NW/OW)		Age (years)		No. of test meals	$EI_{test\ meal}$ (MJ)	Duration of postprandial period (min)
		Mean	SD	Mean	SD			
Flint *et al.* (1998)	19/0	22·8	1·3	24·5	2·8	1	3·0–3·5	270
Flint *et al.* (2001)	0/18	–/33·7	–/2·4	42·6	9·0	1	2·3–3·1	270
Verdich *et al.* (2001)	12/19	23·1/35·8	1·3/3·4	34·7	9·4	1	2·5	180
Bakhøj *et al.* (2003)	9/2	22·3/26·8	1·5/2·0	25·3	2·4	4	1·2–1·5	180
Raben *et al.* (2003)*	9/1	21·1/25·7	1·2/–	22·5	1·4	4	2·5 (♀), 3·0 (♂)	315
Flint *et al.* (2004*a*)	27/1	22·4/26·6	1·5/–	24.	2·6	10	1·1–3·0	180
Flint *et al.* (2004*b*)	16/3	22·5/26·3	1·1/1·1	26·3	0·9	1	2·6–3·3	300

NW, normal weight; OW, overweight; $EI_{testmeal}$, energy intake in the test meal.
*This study included five women who were all normal weight.

seventh study, a test meal was given once to the NW participants and twice to the OW participants, namely before and after a 6-month weight loss period (Verdich *et al.* 2001*b*).

All studies were approved by the Municipal Ethical Committee of Copenhagen and Frederiksberg, Denmark, and all the participants gave informed written consent before entering the studies.

Plasma concentrations of insulin and blood glucose

In all seven studies, venous blood was drawn and analysed for plasma glucose and serum insulin. Serum insulin concentrations were measured using radioimmunoassay according to the principles described by Albano *et al.* (1972). Plasma glucose concentrations were measured by standard enzymatic methods on a Cobas Mira (Roche Diagnostic Systems, Basel, Switzerland) (Deeg *et al.* 1980).

Subjective appetite ratings and ad libitum *energy intake*

In all seven studies, the participants rated their hunger and satiety using a VAS (Flint *et al.* 2000). The VAS were 100 mm long horizontal lines with words anchored at each end, expressing the most negative and most positive rating (for hunger these were: 'I am not hungry at all' and 'I have never been more hungry'). The participants were asked to make a univocal mark on the horizontal line that corresponds to their feelings.

In six of the studies (Flint *et al.* 1998, 2001, 2004*a*,*b*; Verdich *et al.* 2001*b*; Raben *et al.* 2003), participants were served an *ad libitum* lunch and told to eat until comfortably satisfied. In four of the studies, the *ad libitum* meal was a hot-pot consisting of pasta and meat sauce with vegetables (Flint *et al.* 1998, 2001, 2004*b*; Raben *et al.* 2003). In the two remaining studies, it was a cold pasta salad with vegetables, ham and dressing (Verdich *et al.* 2001*b*; Flint *et al.* 2004*a*). The macronutrient composition of the *ad libitum* meals was quite similar, with 13–16 E% (percentage of energy) from protein, 48–50 E% from carbohydrates and 35–37 E% from fat. The *ad libitum* EI ($EI_{ad\ libitum}$) was measured by weighing and calculations based on the assumption of a homogenous distribution of energy and macronutrients in the meal.

Summary measures

Incremental area under the curve (iAUC) was calculated using the trapezoid rule leaving out negative values. iAUC was used as a summary measure for the variables insulin ($iAUC_{insulin}$), blood glucose ($iAUC_{glucose}$) and satiety ($iAUC_{satiety}$). For hunger, the incremental area over the curve ($iAOC_{hunger}$) under the fasting level was used. This was done because the fasting level of hunger is most often higher than the levels of hunger during the postprandial period. Thus, calculating iAUC for hunger would lead to negative values. Furthermore, we used two additional variables for both insulin and blood glucose, namely fasting concentrations ($FAST_{insulin}$, $FAST_{glucose}$) and concentrations immediately before the *ad libitum* meal ($END_{insulin}$, $END_{glucose}$).

Statistics

Meta-analysis is a statistical technique used to integrate the results from separate studies (Alderson *et al.* 2003*a*). In this study, two different types of meta-analysis were conducted.

Weighted fixed effects study level (SL) meta-regression analyses models were chosen for the primary analyses because outcomes were measured in a standard way across studies. The mean effect size and variance were calculated for each study. Studies were then weighted according to the reciprocal of their variance, thereby giving more weight to the studies with the narrower confidence intervals. The SL analyses were conducted in order to examine whether potential effects of insulin and blood glucose on appetite sensations and *ad libitum* EI were consistent across studies. SL analyses were conducted on all participants (ALL) and as subgroup analyses on NW and OW participants separately. The analyses were conducted with $iAUC_{satiety}$, $iAOC_{hunger}$ and $EI_{ad\ libitum}$ as evaluated outcomes. Furthermore, the evaluated covariates were $iAUC_{insulin}$, $iAUC_{glucose}$, $EI_{test\ meal}$, AGE and duration of the postprandial period. SL sensitivity analysis was conducted to test the effect of leaving out the five female participants. Furthermore, additional SL analyses were conducted to test the effect of $FAST_{insulin}$, $FAST_{glucose}$, $END_{insulin}$ and $END_{glucose}$. All the SL analyses were univariate due to the limited number of studies. Furthermore, all the SL analyses were conducted using the fixed effects approach based on the assumption that only intra-study variations affected the results. However, to examine the degree of heterogeneity (inter-study variation) and stability, and thus whether the choice of using fixed effects models was reasonable, random effects SL analyses were also performed. This random effects approach is based on the assumption that both intra- and inter-study variations affect the results of the analyses.

To evaluate the effect of potential confounding factors, univariate and multivariate regression analyses with individual participant data (IPD) were performed. In order to perform IPD analyses, raw data from each of the included studies were collected. In the IPD analyses, the data were treated as if they originated from a single study. The IPD analyses were conducted on ALL participants, and as subgroup analyses on NW and OW. The evaluated outcomes were $iAUC_{satiety}$, $iAOC_{hunger}$ and $EI_{ad\ libitum}$. Furthermore, $iAUC_{insulin}$, $iAUC_{glucose}$, AGE, $EI_{test\ meal}$ and the duration of the postprandial period were included as covariates.

Apart from the meta-analyses, a Student's *t* test was conducted to examine whether there was a difference between the NW and OW with respect to insulin and blood glucose concentrations, satiety, hunger and $EI_{ad\ libitum}$. Furthermore, a Spearman correlation analysis was performed to test whether congruity existed between the VAS scores and the subsequent $EI_{ad\ libitum}$.

All the statistical analyses in the present report were conducted using STATA (version 8·0 for Windows, Stata Corporation, TX, USA). In order to reduce the risk of bias, all statistical analyses were pre-specified. All the continuous variables except BMI were ln-transformed. BMI was dichotomised with a cut-off value of 25, creating two categories classified as NW and OW, respectively. In some of the

included studies, the participants received more than one test meal. Mean values were used for these participants, so that each participant was included only once in the analyses. Mean values were also used in one of the studies, where the participants received identical meals before and after a 6-month weight loss period (Verdich *et al.* 2001*b*). The results of the SL and IPD analyses were expressed by regression coefficients with corresponding SE and level of significance (*P*). The level of significance was set to $P<0{\cdot}05$.

Results

Insulin and short-term appetite regulation

The SL analysis including ALL participants showed that $iAUC_{insulin}$ was positively correlated with $iAOC_{hunger}$, meaning that a larger postprandial $iAUC_{insulin}$ is associated with smaller increases in sensations of hunger (Table 2). However, only tendencies were seen for $iAUC_{insulin}$ to be correlated with $iAUC_{satiety}$ and $EI_{ad\ libitum}$ for ALL participants (Table 2). According to the subgroup SL analyses of NW, $iAUC_{insulin}$ was positively correlated with $iAUC_{satiety}$ and $iAOC_{hunger}$, but not associated with $EI_{ad\ libitum}$ (Fig. 1). This indicates that increased insulin levels in the NW group are associated with increased sensations of satiety and decreased sensations of hunger. The subgroup SL analyses of OW showed that $iAUC_{insulin}$ was positively correlated with $iAOC_{hunger}$ (Fig. 2) and inversely correlated with $EI_{ad\ libitum}$. Thus, the larger the $iAUC_{insulin}$, the smaller the sensations of hunger and the smaller the subsequent $EI_{ad\ libitum}$. The sensitivity SL analysis without the female participants showed results similar to the SL analysis including ALL participants, with $iAUC_{insulin}$ being positively correlated to $iAOC_{hunger}$ (Table 2). Additional SL analyses showed that $FAST_{insulin}$ did not correlate with $iAUC_{satiety}$, $iAOC_{hunger}$ or $EI_{ad\ libitum}$ ($P\geq0{\cdot}3$). Likewise, $END_{insulin}$ did not correlate with $EI_{ad\ libitum}$ ($P>0{\cdot}1$).

The univariate IPD analysis including ALL participants showed that $iAUC_{insulin}$ was positively correlated to both $iAUC_{satiety}$ and $iAOC_{hunger}$, and inversely associated with $EI_{ad\ libitum}$ (Table 3). However, when tested in multivariate IPD analysis with ALL participants, only the inverse correlation between $iAUC_{insulin}$ and $EI_{ad\ libitum}$ remained significant. In the univariate subgroup IPD analysis, including NW $iAUC_{insulin}$ was positively correlated to $iAUC_{satiety}$ and $iAOC_{hunger}$, and inversely correlated to $EI_{ad\ libitum}$. When tested in multivariate analysis (adjusted for $iAUC_{glucose}$, AGE, $EI_{testmeal}$ and the duration of the postprandial period), $iAUC_{insulin}$ was correlated to all three outcomes in NW. According to the univariate subgroup IPD analysis, including OW participants' $iAUC_{insulin}$ was only inversely correlated to $EI_{ad\ libitum}$. However, no associations were seen between $iAUC_{insulin}$ and the outcomes when tested in multivariate analysis including OW participants.

Blood glucose and short-term appetite regulation

The SL analysis including ALL participants showed that $iAUC_{glucose}$ was not associated with any of the three outcomes (Table 2). Likewise, when tested in sensitivity SL analysis without women, $iAUC_{glucose}$ was still not correlated with any of the outcomes. The subgroup SL analyses for NW and OW did not show any associations between $iAUC_{glucose}$ and the outcomes. Additional SL analyses showed that $FAST_{glucose}$ did not correlate with $iAUC_{satiety}$, $iAOC_{hunger}$ or $EI_{ad\ libitum}$ ($P>0{\cdot}8$). Likewise, $END_{glucose}$ was not associated with $EI_{ad\ libitum}$ ($P>0{\cdot}2$).

Univariate IPD analysis including ALL participants showed that $iAUC_{glucose}$ was positively correlated to $iAOC_{hunger}$ and inversely correlated to $EI_{ad\ libitum}$ (Table 3). However, when tested in a multivariate analysis, only the inverse association between $iAUC_{glucose}$ and $EI_{ad\ libitum}$ remained significant. According to the univariate subgroup IPD analysis, including NW $iAUC_{glucose}$ was only positively correlated to $iAOC_{hunger}$, but when tested in multivariate analysis no associations at all were seen. Univariate subgroup IPD analysis including OW showed that $iAUC_{glucose}$ correlated inversely to $EI_{ad\ libitum}$.

Table 2. Study level meta-regression analyses: effects of insulin and glucose levels on satiety, hunger and *ad libitum* energy intake for all, normal weight, overweight male participants

	$iAUC_{satiety}$			$iAOC_{hunger}$			$EI_{ad\ libitum}$		
Analysis	Coefficient	SE	*P*	Coefficient	SE	*P*	Coefficient	SE	*P*
$iAUC_{insulin}$									
ALL	0·26	0·13	0·074	0·31	0·09	0·008	−0·14	0·07	0·084
NW	0·76	0·13	0·004	0·73	0·19	0·018	0·17	0·20	0·463
OW	0·21	0·20	0·351	0·33	0·07	0·012	−0·29	0·07	0·022
Sensitivity	0·26	0·14	0·099	0·32	0·10	0·011	−0·12	0·07	0·130
$iAUC_{glucose}$									
ALL	0·31	0·28	0·291	0·39	0·30	0·224	−0·26	0·15	0·146
NW	0·39	0·46	0·437	0·62	0·40	0·202	0·39	0·39	0·391
OW	0·28	0·43	0·548	0·70	0·41	0·168	−0·54	0·22	0·086
Sensitivity	0·30	0·29	0·335	0·40	0·32	0·243	−0·21	0·13	0·159

ALL, all participants; NW, normal weight participants; OW, overweight or obese participants; $iAUC_{insulin}$, $iAUC_{glucose}$ and $iAUC_{satiety}$, incremental area under the insulin, glucose and satiety response curves; $iAOC_{hunger}$, incremental area over the hunger response curve; $EI_{ad\ libitum}$, *ad libitum* energy intake.

Data are expressed as regression coefficients with their standard error and corresponding level of significance (*P*). Level of significance was $P<0{\cdot}05$, Sensitivity analyses were conducted on male participants only.

Age, duration of the postprandial period and energy intake in the test meal were included as covariates in the analyses. Furthermore, $iAUC_{glucose}$ was included as a covariate in the analyses with $iAUC_{insulin}$ as independent variable, and vice versa.

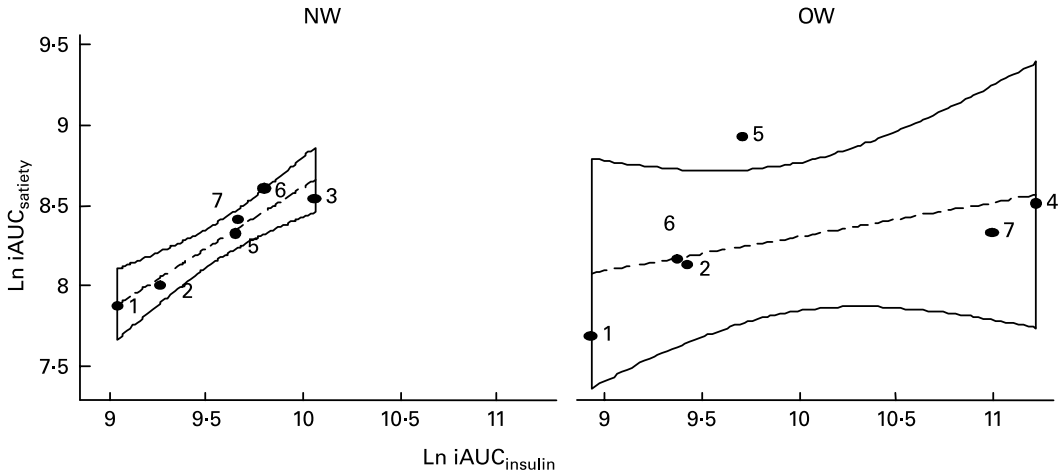

Fig. 1. Scatter plot of relationships between postprandial insulin concentrations and ratings of satiety for normal weight (NW) and overweight (OW) participants. The associations shown in this figure are: NW ($r = 0{\cdot}76$; $P = 0{\cdot}004$) and OW ($r = 0{\cdot}21$; $P = 0{\cdot}351$). iAUC$_{insulin}$ and iAUC$_{satiety}$, incremental area under the insulin and satiety response curves; filled circles represent point estimates from the individual studies (reference numbers next to the circles refer to study numbers: 1, Bakhoj *et al.* 2003; 2, Flint *et al.* 2004*a*; 3, Flint *et al.* 1998; 4, Flint *et al.* 2001; 5, Flint *et al.* 2004*b*; 6, Raben *et al.* 2003; 7, Verdich *et al.* 2001*b*), whereas the slopes of the lines represent the regression coefficients with corresponding 95 % confidence intervals. The values on the *x*- and *y*-axes are not absolute values, but instead the values that resulted from the ln transformation of the data.

However, the multivariate subgroup IPD analysis found no associations between iAUC$_{glucose}$ and the three outcomes.

The *P*-values and confidence intervals did not differ markedly between the random effects and fixed effects models in the meta-regression analyses, meaning that the heterogeneity seems small and therefore using the fixed effects models for the SL meta-regression analyses seems reasonable.

The *t*-test showed that OW participants had significantly larger levels of iAUC$_{insulin}$ ($P = 0{\cdot}001$) and iAUC$_{glucose}$ ($P = 0{\cdot}001$) than their NW counterparts. However, NW participants had a larger EI$_{ad\ libitum}$ than OW participants ($P = 0{\cdot}028$).

Finally, the Spearman correlation analyses showed that for NW participants, EI$_{ad\ libitum}$ correlated with iAUC$_{satiety}$ ($r = -0{\cdot}2234$, $P = 0{\cdot}045$) and iAOC$_{hunger}$ ($r = -0{\cdot}2476$, $P = 0{\cdot}028$). However, for ALL and OW participants no correlations were found.

Discussion

The results of the present meta-analysis suggest that insulin, but not blood glucose, might be an important satiety signal in short-term appetite regulation in NW, but not in OW participants.

The SL analysis including ALL participants showed that the insulin response was significantly and inversely related to hunger and, although this association was strong, only tendencies were seen between the insulin response, and satiety and *ad libitum* EI. However, according to our

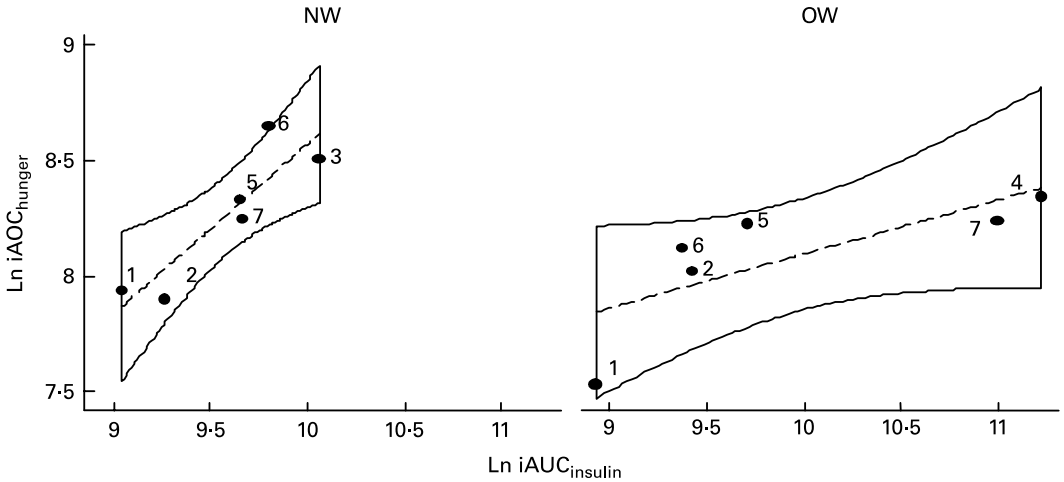

Fig. 2. Scatter plot of relationships between postprandial insulin concentrations and ratings of hunger for normal weight (NW) and overweight (OW) participants. The associations shown in this figure are: NW ($r = 0{\cdot}73$; $P = 0{\cdot}018$) and OW ($r = 0{\cdot}33$; $P = 0{\cdot}012$). iAUC$_{insulin}$, incremental area under the insulin response curve; iAOC$_{hunger}$, incremental area over the hunger response curve. The filled circles represent point estimates from the individual studies (reference numbers next to the circles refer to study numbers: 1, Bakhoj *et al.* 2003; 2, Flint *et al.* 2004*a*; 3, Flint *et al.* 1998; 4, Flint *et al.* 2001; 5, Flint *et al.* 2004*b*; 6, Raben *et al.* 2003; 7, Verdich *et al.* 2001*b*), whereas the slopes of the lines represent the regression coefficients with corresponding 95 % confidence intervals. The values on the *x*- and *y*-axes are not absolute values, but instead the values that resulted from the ln transformation of the data.

Table 3. Univariate and multivariate individual participant data regression analyses: effects of insulin and glucose levels on satiety, hunger and *ad libitum* energy intake for all, normal weight and overweight participants

	$iAUC_{satiety}$			$iAOC_{hunger}$			$EI_{ad\ libitum}$		
Analysis	Coefficient	SE	*P*	Coefficient	SE	*P*	Coefficient	SE	*P*
$iAUC_{insulin}$									
Univariate									
ALL	0·27	0·09	0·003	0·24	0·10	0·024	−0·18	0·04	<0·001
NW	0·66	0·19	0·001	0·88	0·21	<0·001	−0·16	0·08	0·045
OW	0·08	0·13	0·528	0·11	0·17	0·530	−0·18	0·09	0·044
Multivariate									
ALL	0·12	0·13	0·354	0·16	0·15	0·305	−0·13	0·05	0·016
NW	0·60	0·27	0·027	0·88	0·28	0·003	−0·32	0·10	0·001
OW	−0·03	0·19	0·878	−0·13	0·10	0·202	−0·16	0·24	0·948
$iAUC_{glucose}$									
Univariate									
ALL	0·17	0·10	0·093	0·23	0·11	0·045	−0·18	0·04	<0·001
NW	0·23	0·14	0·112	0·40	0·16	0·012	−0·09	0·05	0·113
OW	−0·03	0·14	0·829	0·02	0·19	0·906	−0·24	0·08	0·003
Multivariate									
ALL	0·07	0·11	0·520	0·18	0·13	0·167	−0·10	0·05	0·032
NW	0·14	0·14	0·358	0·23	0·16	0·156	−0·06	0·05	0·268
OW	−0·02	0·17	0·903	−0·17	0·09	0·073	0·02	0·23	0·948

ALL, all participants; NW, normal weight participants; OW, overweight or obese participants; $iAUC_{insulin}$, $iAUC_{glucose}$ and $iAUC_{satiety}$,- incremental area under the insulin, glucose and satiety response curves; $iAOC_{hunger}$, incremental area over the hunger response curve; $EI_{ad\ libitum}$, *ad libitum* energy intake.

Results are listed as regression coefficients with their standard error and corresponding level of significance (*P*). Level of significance was $P<0·05$. Multivariate analyses included $iAUC_{insulin}$, $iAUC_{glucose}$, AGE, $EI_{test\ meal}$ and the duration of the postprandial period as covariates.

Age, duration of the postprandial period and energy intake in the test meal were included as covariates in the multivariate analyses. Furthermore, $iAUC_{glucose}$ was included as a covariate in the multivariate analyses with $iAUC_{insulin}$ as independent variable, and *vice versa*.

subgroup IPD analyses, it seems that these somewhat inconsistent findings from the SL analyses might be due to the pooling of participants with a broad range in BMI, since the subgroup multivariate IPD analyses consistently showed that the insulin response was associated with all three outcomes for NW (e.g. hunger; $r = 0·88$), but not for OW participants.

To our knowledge, this is the first meta-analysis of these questions of interest. Only a few smaller studies have examined differences between NW and OW individuals with respect to the associations between insulin and short-term appetite sensations (Rodin *et al.* 1988; Speechly & Buffenstein, 2000; Verdich *et al.* 2001*b*). In the study by Verdich *et al.* (2001*b*), fasting insulin concentrations, total and incremental area under the insulin response curve and the insulin concentrations immediately before the *ad libitum* meal all correlated inversely with *ad libitum* EI in lean, but not in obese participants (Verdich *et al.* 2001*b*). Likewise, the insulin concentrations immediately before the *ad libitum* meal also correlated inversely with *ad libitum* EI in lean, but not in obese participants in the second study (Speechly & Buffenstein, 2000). In contrast, a third study showed that insulin concentrations at 15, 30 and 45, but not at 95, 135 and 155 min after a test meal were positively correlated with subsequent *ad libitum* EI in obese, but not in lean participants (Flint *et al.* 2000). In our present meta-analysis, the integrated postprandial insulin response seemed the best predictor of satiety and subsequent *ad libitum* EI, since it was correlated with the outcomes, as opposed to the fasting insulin concentrations and the insulin concentrations immediately before the *ad libitum* meal.

If insulin does act as a short-term satiety signal, then several mechanisms could be responsible for this effect. First of all, insulin could work directly through insulin receptors in the hypothalamic area, as suggested by Verdich *et al.* (2001*b*). Extensive evidence supports a role for central insulin receptors in the regulation of appetite (Brüning *et al.* 2000; Porte *et al.* 2002). However, the central effect of insulin could also be mediated indirectly through interaction with other satiety-inducing peptides, e.g. cholecystokinin (CCK), GLP-1 and peptide YY (PYY) (Figlewicz *et al.* 1995; Schwartz & Morton, 2002; Flint *et al.* 2004*a*). Another possibility is that insulin affects the intracellular level of ATP in the liver by changing the transport of energy-yielding substrates into the liver. Several studies have proposed that glucose sensors and other metabolic sensors in the liver relay information about the ATP level in the liver through afferent nerves to the brain and thereby change food intake (Friedman, 1995; Langhans, 1996). On the other hand, the results of insulin infusion studies do not support a direct satiating effect of insulin (Woo *et al.* 1984; Chapman *et al.* 1998; Gielkens *et al.* 1998). However, this may be due to the fact that when insulin is infused intravenously, glucose needs to be infused concomitantly to prevent hypoglycaemia. Consequently, the potential effect of insulin on the energy status of the liver is bypassed and therefore these infusion studies do not reflect normal *in vivo* circumstances.

The observed differences in insulin's association with short-term appetite regulation between NW and OW participants might be due to decreased central insulin sensitivity in the OW participants, as suggested by Verdich *et al.* (2001*b*). This is supported by a study showing that genetic deletion of the brain insulin receptor causes central insulin resistance and obesity in rodents (Brüning *et al.* 2000). Further, such decreased central insulin sensitivity could be similar to the decreased peripheral insulin sensitivity, which is a

well-known complication of the obese state (Bonadonna *et al.* 1990). In our study, the OW participants had significantly larger $iAUC_{insulin}$ and $iAUC_{glucose}$ than the NW participants, which supports the hypothesis of decreased insulin sensitivity, hyperinsulinaemia and hyperglycaemia in these participants. However, so far this is rather speculative and thus other mechanisms might be involved. Nevertheless, given that a potential satiating effect of insulin is reduced or diminished in the OW participants, it seems surprising that NW participants had a larger *ad libitum* EI than the OW participants. Our findings might be explained by the fact that several other mechanisms (e.g. psychological, sensory and cognitive) are involved in the regulation of appetite (Blundell, 1999) and thus might compensate for a reduced satiating effect of insulin and thereby explain the smaller *ad libitum* EI in the OW participants. In addition, the OW participants in our study might have been more affected by the experimental settings than the NW participants and therefore the *ad libitum* EI in the OW participants might not have reflected how hungry this group actually was. Furthermore, OW subjects might not be as good as NW subjects in sensing their hunger. This is supported by the results of the correlation analysis, which showed that in NW, but not in OW participants, sensations of hunger and satiety correlate with *ad libitum* EI.

The lack of association between insulin and appetite for OW subjects could be due to lack of statistical power in this group. However, although the analyses included more than twice as many NW as OW subjects, the sample size of the OW group was still large (n 44). Thus, it seems unlikely that lack of power was the reason for the lack of associations in the OW subjects

Only five of the subjects were female, which means that this group was too small for conducting subgroup analyses. In combination with the fact that the results of the analyses were unchanged by excluding the women, this therefore suggests that the results of this meta-analysis are only applicable to men.

According to the SL analyses, the blood glucose response was not related to any of the outcomes. Furthermore, only the multivariate IPD analysis including all participants revealed an inverse association between the blood glucose response and *ad libitum* EI. Thus, these results do not support a significant role for blood glucose in short-term appetite regulation. This is supported by other studies examining the role of blood glucose in relation to appetite sensations and EI (Lavin *et al.* 1996; Porte *et al.* 2002). However, it has been suggested that the potential effect of insulin as a satiety signal might require raised blood glucose concentrations (Chapman *et al.* 1998). Nevertheless, in our meta-analysis, the associations between the insulin response and the outcomes do not seem to be due to blood glucose. On the other hand, a number of previous studies have suggested that a decline in blood glucose concentrations precedes meal initiation (Campfield & Smith, 1990; Smith & Campfield, 1993). Our study showed no correlation between the blood glucose concentrations immediately before the *ad libitum* meal and *ad libitum* EI. However, due to the fixed timing of the protocols, this does not necessarily exclude a role for blood glucose in meal initiation. Thus, whether this pre-meal decline in blood glucose actually is a causal factor in meal initiation is still controversial (De Graaf *et al.* 2004).

Even though the correlations between the insulin response and the three outcomes observed in the NW subjects did not depend on the examined covariates, this does not exclude that other potential confounding factors might have been responsible for these correlations. The most obvious potentially confounding factors seem to be the incretin hormones, glucose-dependent insulinotropic polypeptide and GLP-1. GLP-1 has been proposed to have a satiating effect, which does not depend on its effects on insulin (Lavin *et al.* 1998). Other studies have supported the hypothesis of a satiety-promoting effect of GLP-1 (Verdich *et al.* 2001*a*), whereas glucose-dependent insulinotropic polypeptide does not seem to influence appetite sensation (Flint *et al.* 2004*b*). However, one study found that insulin concentrations did not correlate with incretin hormones, but still explained 67 % of the variations between *ad libitum* EI in lean participants (Verdich *et al.* 2001*b*). Thus, further studies are required to clarify how insulin and the incretin hormones interact in the regulation of appetite.

A common problem when conducting several statistical analyses is that the risk of mass significance (type I errors) increases as the number of subgroup analyses and characteristics examined increases (Deeks *et al.* 2003). In this meta-analysis, six different subgroup analyses, each including three different outcomes and 2–5 covariates, were conducted. Therefore, it is possible that the analyses might have yielded false-positive results. However, several precautions were taken to reduce the risk of mass significance, including conducting sensitivity analyses and pre-specifying all analyses. A possible approach to reduce the risk of mass significance is to adjust the level of significance from $P<0{\cdot}05$ to $P<0{\cdot}01$ or $P<0{\cdot}001$, and thereby increase the demands for statistical significance (Alderson *et al.* 2003*b*). If we in this study set the level of statistical significance to $P<0{\cdot}01$, the overall results and conclusions, especially those pertaining to insulin, do not change. Thus, it seems unlikely that mass significance was responsible for the consistent findings of significant correlations between insulin and the three outcomes in this meta-analysis.

A limitation of this meta-analysis is that all the included studies were conducted at the Department of Human Nutrition in Copenhagen, which increases the risk of our inclusion criteria being biased (Egger & Smith, 1998). Furthermore, the seven included studies were all rather small (n10–31). However, to our knowledge, only a few studies apart from those included in our meta-analysis have examined the roles of insulin (and blood glucose) in short-term appetite regulation using this methodology, and these studies were also rather small (n12–38; Speechly & Buffenstein, 2000; Alderson *et al.* 2003*a*). Thus, our meta-analysis might be one of the most comprehensive studies to address this subject. Finally, the problem with the interpretation of the results of this meta-analysis is that the present design is an observational *post hoc* analysis where it is not possible to draw any conclusions about the causality of the associations (Thompson & Higgins, 2002). Nevertheless, in line with the study by Verdich *et al.* (2001*b*), we suggest that insulin might be a mediator of satiety sensations in the postprandial period, and thereby decrease *ad libitum* EI in NW participants.

Conclusion

In summary, our results suggest that insulin, but not glucose, is associated with short-term appetite regulation in healthy NW participants, and that the association seems to weaken or disappear when body weight increases. We conclude that the postprandial insulin response may be an important satiety signal, and propose that central nervous system insulin resistance in overweight individuals might explain the blunted effect on appetite.

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