Animal (2007), **1:6**, pp 865–871 © The Animal Consortium 2007
doi: 10.1017/S1751731107000018

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Behaviour influences cholesterol plasma levels in a pig model

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(Received 30 November 2006; Accepted 19 March 2007)

Little is known about the relationship between feed intake behaviour and cholesterol levels in humans. This can be attributed to the fact that feed intake behaviour in humans is difficult to assess. The relationships between feed intake, feed efficiency and feed intake behaviour, and cholesterol and triglyceride levels were investigated at an average age of 187 days, in a pig model consisting of 202 Duroc barrows. Feed intake and feed intake behaviour were recorded individually and daily by means of an electronic identification system. Animals with high levels of total cholesterol also had high levels of high-density lipoprotein (HDL), low-density lipoprotein (LDL) cholesterol and triglycerides. Animals with high levels of HDL also had high levels of LDL and triglycerides, and animals with high levels of LDL also had high levels of triglycerides. Animals with higher BW, higher backfat thickness, higher BW gain, higher gain of backfat deposition, higher feed intake, higher residual feed intake (RFI) and higher feed intake rate had higher levels of total, HDL and LDL plasma cholesterol. Results indicate that the relationship between feed intake and cholesterol levels is a long-term relationship, while the relationship between RFI and cholesterol levels is more of a short-term nature. The relationship between intake rate and cholesterol plasma levels disappeared after correction for the amount of feed consumed. Results indicate that feed intake independent of metabolic BW, growth and fatness, i.e. 'RFI', was positively correlated with cholesterol plasma levels. This suggests that eating food over and above the maintenance and growth requirements constitutes a health risk independent of the level of fatness.

Keywords: cholesterol, feed intake behaviour, pig model, sire effect, triglyceride

Introduction

The effect of the diet on cholesterol levels and cardiovascular disease has been widely investigated (Stanner, 2006). However, little is known about the relationship between feed intake behaviour – such as total feed intake, intake rate, frequency and duration – and cholesterol levels in humans. This can be attributed to the fact that, in humans, feed intake behaviour is difficult to assess because it is influenced by a large variety of variables, such as physiological and genetic traits, psychological and socio-cultural factors, experiences, environmental conditions, and personal preferences, habits and sensitivities (De Castro, 1999).

The pig is considered to be the most suitable non-primate animal model since it resembles the human situation better than any other non-primate animal species with regard to eating behaviour, anatomy and physiology of the gastrointestinal tract (Davis *et al.*, 2001). Also, the pig has become increasingly accepted as an animal model for research on cholesterol and lipoprotein metabolism (Chapman and Goldstein, 1976; Pond and Mersmann, 1996).

In humans, the study of the relationship between food intake patterns and cholesterol levels mainly investigates situations in which food intake is related to obesity (e.g. Cordero-MacIntyre *et al.*, 2000; Nicklas *et al.*, 2001) and food intake patterns are related to food quality (e.g. Van Dam *et al.*, 2003; Scaglioni *et al.*, 2004). In animals, and farm animals, in particular, much research is directed to investigation of feed efficiency traits. A widely used estimate is that of 'residual feed intake (RFI)', which is defined as the feed that is consumed by an individual and its consumption as predicted from a model involving its maintenance and growth requirements (Luiting, 1990; Rauw *et al.*, 2006a). Because of the complexity of measuring actual food intake in humans, this concept has never been applied to human research.

The aim of the present study is to investigate the relationship between feed intake behaviour and cholesterol and triglyceride plasma levels in a pig model.

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Material and methods

Animals and experimental procedures

Animals were provided by the company Selección Batallé (Riudarenes, Girona, Spain). The data set consisted a total of 202 Duroc barrows in two replicates (99 and 103 animals, respectively). Animals were born between August and September 2003 (replicate 1) and between March and April 2004 (replicate 2). Barrows were castrated sons of five sires (17, 34, 40, 42 and 69 sons per sire, respectively) and 202 dams born on three farms (60, 69 and 73 animals, respectively). Sire 1 had offspring in the first replicate only because he died before dams of the second replicate could be inseminated. There was no particular reason for the fact that sire 5 had almost twice as many offsprings as the others.

At weaning (11–23 days of age), pigs were moved to the test station 'Centre de Control Porci' (CCP-IRTA, Monells, Girona, Spain), distributed over 16 pens in four rooms and all were subjected to the same management procedures. At this stage, animals were housed based on age; animals that belonged to this experiment were mixed with animals that did not take part in this experiment, possibly of different breeds and sex.

Plasma cholesterol (total, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol) and triglyceride levels were measured once between 29 and 55 days of age (43 ± 0.5 days of age; $TOTAL_{43}$, HDL_{43}, LDL_{43} and $TRIG_{43}$, respectively), and in the same individuals once between 171 and 201 days of age (187 ± 0.6 days of age; $TOTAL_{187}$, HDL_{187}, LDL_{187} and $TRIG_{187}$, respectively). At about 43 days of age, blood was collected from the jugular vein without fasting the animals. The second blood samples were taken at slaughter after the animals were fasted for over 12 h.

Venous blood was collected into EDTA-containing tubes. Plasma total cholesterol and triglycerides were determined by a Technicon Chem 1 assay (Technicon Instruments, Tarrytown, NY), and HDL was measured in the supernatant after precipitation of apoB-containing lipoproteins with heparin–manganese chloride. The LDL concentration was calculated with the equation of Friedewald *et al*. (1972) as stated in Pond *et al*. (1997). Total plasma cholesterol includes HDL, LDL, intermediate-density lipoproteins, very low-density lipoproteins (VLDL) and chylomicrons, of which LDL constitutes the largest fraction and HDL the next largest fraction (Pond *et al*., 1993).

Between 2 and 3 months after birth, pigs participating in the present study were moved to the fattening and control unit where they were distributed over 10 pens (five pens at each side of a central corridor in the same barn) in groups of 8–12 animals. Approximately 2 weeks later and for 115 (replicate 1) and 118 (replicate 2) days, feed intake (FI; kg/day), intake time (TIME; min/day) and intake frequency (FREQ; visits/day) were recorded individually and daily by means of an electronic identification system (HOKOFARM, IVO-G®; Marknesse, The Netherlands). The feeding station consisted of a single-space feed hopper, a trough which was weighted continuously and an electronic identification system that was activated by ear responders as the animal entered the station. The feeding station was connected through a load cell to a computer and the trough was refilled if the amount of feed left after a visit of a pig was completed was below 10 kg. At each visit of a pig to the feeder, time and weight of the feed at the beginning and at the end of the visit were recorded automatically, together with the animal identification number. FI per visit was calculated as the difference between amounts recorded just before and after the visit, with an accuracy of 10 g. Intake rate (RATE; kg/min) was estimated for each individual by dividing FI by intake time. Intake rate is thus the amount of feed ingested per minute spent eating (Rauw *et al*., 2006b). On the 1st day of the feeding trial, animals were between 73 and 95 days of age.

Because of electricity failures, 24 recordings were missing in replicate 1, and 16 in replicate 2, spread out over the trial period. Missing values were estimated according to Rauw *et al*. (2006a). For the present study, FI traits were considered for the period between 170 and 175 days of age ($FI_{170–175}$, $TIME_{170–175}$, $FREQ_{170–175}$ and $RATE_{170–175}$). This period was closest to the second age at which cholesterol and triglyceride levels were measured; data were available for 179 of the 202 barrows. Choosing a period at later ages would have decreased the number of animals considerably.

Until an average of 155 days of age, pigs were fed *ad libitum* on a standard diet with (per kg) 180 g crude protein (CP), 38 g fibre, 70 g fat, 10 g lysine and 3 g methionine. Afterwards, animals were fed *ad libitum* on a standard diet with (per kg) 151 g CP, 45 g fibre, 49 g fat, 9 g lysine and 3 g methionine. The net energy concentration of the diets was 10 290 and 9975 kJ/kg, respectively. Feed ingredients are given in Table 1. The amount of cholesterol in the feed was not specifically indicated. Only very small amounts would have been present because of the small amount of animal fat.

BW and backfat thickness (BFT) were recorded at the average age of 85, 99, 122, 150, 171 and 189 days of age in replicate 1 and of 82, 104, 119, 140, 160 and 182 days of age in replicate 2. BFT was measured by the PIGLOG 105 A-mode apparatus (SFK Technology, Soborg, Denmark) as the average of two ultrasonic measurements taken on each side of the spinal column, 5 cm from the mid-dorsal line at the last rib. A linear regression equation was fitted individually to all six measurements on BW and BFT as a function of age, resulting in estimates of body-weight gain (BWG; kg/day) and rate of fat deposition (FATG; mm/day; Rauw *et al*., 2006b). The R^2 of the linear regression was 99.0 ($\pm$0.146%) for BW and 95.9 ($\pm$0.550%) for BFT (Rauw *et al*., 2006b). BW and BFT were estimated at 170 days of age from this linear regression (BW_{170} and BFT_{170}, respectively).

Individual RFI was estimated for the period between 170 and 175 days of age ($RFI_{170–175}$) from a multiple linear regression of $FI_{170–175}$ on estimated values of metabolic BW, BWG and BFT (Rauw *et al*., 2006a), for the

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Table 1 *Main feed ingredients (2% and higher) of the feed given up to an average of 155 days of age (Feed 1) and after this period (Feed 2)*

	%
Feed 1	
Barley	30.0
T Soja	20.1
Maize	15.0
Wheat	11.9
Fibre from rye	10.0
G Soja	5.0
Animal fat	3.8
Feed 2	
Tapioca	21.3
Toasted soya meal	18.4
Peas	13.9
Barley	13.4
Rye	8.9
Maize	5.0
Wheat	5.0
Animal fat	3.8
Bran	3.4
Molasses	2.8
Beet pulp	2.0

corresponding period:

$$FI_i = b_0 + (b_1 \times BW_i^{0.75}) + (b_2 \times BWG_i) + (b_3 \times BFT_i) + e_i, \quad (1)$$

where FI_i = FI of individual i (kg/5 days), $BW_i^{0.75}$ = average metabolic body weight of individual i ($kg^{0.75}$), BWG_i = BW gain of individual i (kg/5 days), BFT_i = average backfat thickness of individual i (mm), b_0 = population intercept, b_1, b_2, b_3 = partial regression coefficients representing maintenance requirements per metabolic kg, feed requirements for growth and feed requirements related to body composition, respectively; e_i = the error term, representing RFI of individual i (kg/period). Metabolic BW and BFT were estimated as the average of the estimated values at the beginning and at the end of the period between 170 and 175 days of age.

To investigate further the relationship between cholesterol measurements, and FI and RFI, in addition, FI was summed and RFI estimated for the periods between 95 and 115, 115 and 135, 135 and 155 and 155 and 175 days of age.

Blood sampling and the recording of feeding traits were approved by the Ethical Committee of the Institution (Institut de Recerca i Technologia Agroalimentàries).

Statistical analysis

The SAS program (Statistical Analysis Systems Institute, 1999) was used for statistical analysis of all traits. The model used to describe the data on $TOTAL_{43}$, HDL_{43}, LDL_{43} and $TRIG_{43}$ was

$$Y_{ijklm} = \mu + Sire_i + Repl_j + Farm_k + PenW(Repl)_l + e_{ijklm}, \quad (2)$$

where μ = population intercept, $Sire_i$ = effect of sire i (1–5), $Repl_j$ = effect of replicate j (1, 2), $Farm_k$ = effect of farm of origin k (1–3), $PenW(Repl)_l$ = effect of pen (until weaning; 1–16) nested within replicate l, and e_{ijklm} = residual error term of animal m, $e_{ijklm} \sim NID(0, \sigma_e^2)$. All effects except the residual error term were considered fixed. The traits $TOTAL_{43}$, HDL_{43}, LDL_{43} and $TRIG_{43}$ were denoted by Y_{ijklm}, as measured on animal m of sire i, born in replicate j, originating from farm k, housed in pen l. Initially, also the effect of age at blood measurement was included in the model, but because this was not significant for any of the four traits, it was excluded from further analysis.

The model used to describe the data on $TOTAL_{187}$, HDL_{187}, LDL_{187} and $TRIG_{187}$ was

$$Y_{ijkl} = \mu + Sire_i + Repl_j + AGE_k + e_{ijkl}, \quad (3)$$

where AGE_k = age at second blood measurement, e_{ijkl} = residual error term of animal l, $e_{ijkl} \sim NID(0, \sigma_e^2)$, and μ, $Sire_i$ and $Repl_j$ are as in model (2). All effects except the residual error term were considered fixed. The traits $TOTAL_{187}$, HDL_{187}, LDL_{187} and $TRIG_{187}$ were denoted by Y_{ijkl}, as measured on animal l of sire i, born in replicate j, measured at age k. Initially, also the effect of pen (fattening period) was included in the model, but because this was not significant for any of the four traits, it was excluded from further analysis.

The model used to describe the data on BW, BFT and FI behaviour was

$$Y_{ijkl} = \mu + Sire_i + Repl_j + PenF(Repl)_k + e_{ijkl}, \quad (4)$$

where μ = population intercept, $PenF(Repl)_j$ = effect of pen (fattening period; 1–10) nested within replicate j, e_{ijkl} = residual error term of animal m, $e_{ijkl} \sim NID(0, \sigma_e^2)$, and $Sire_i$ and $Repl_j$ are as in model (2). All effects except the residual error term were considered fixed. The traits tested under this model were denoted by Y_{ijkl}, as measured on animal l of sire i born in replicate j, housed in pen k: BW_{170}, BFT_{170}, $FI_{170–175}$, $RFI_{170–175}$, $TIME_{170–175}$, $FREQ_{170–175}$, $RATE_{170–175}$, BWG and FATG. Initially, the effects of farm of origin and trial day were also included in the model, but because this was not significant for any of the nine traits, it was excluded from further analysis.

Phenotypic correlations were calculated after adjusting the values for the effects of replicate, farm and pen ($TOTAL_{43}$, HDL_{43}, LDL_{43} and $TRIG_{43}$), for replicate and age at measurement ($TOTAL_{187}$, HDL_{187}, LDL_{187} and $TRIG_{187}$), and for the effects of replicate and pen (BW_{170}, BFT_{170}, $FI_{170–175}$, $RFI_{170–175}$, $TIME_{170–175}$, $FREQ_{170–175}$, $RATE_{170–175}$, BWG and FATG).

Results

Table 2 presents mean values and standard errors for $TOTAL_{43}$, HDL_{43}, LDL_{43}, $TRIG_{43}$, $TOTAL_{187}$, HDL_{187}, LDL_{187}, $TRIG_{187}$, BW_{170}, BFT_{170}, BWG, FATG, $FI_{170–175}$, $RFI_{170–175}$, $TIME_{170–175}$, $FREQ_{170–175}$ and $RATE_{170–175}$. Phenotypic

https://doi.org/10.1017/S1751731107000018 Published online by Cambridge University Press

Table 2 *Mean values and standard errors (s.e.) for total ($TOTAL_i$)†, high-density lipoprotein (HDL_i)† and low-density lipoprotein (LDL_i)† cholesterol and triglyceride levels ($TRIG_i$)†, body weight (BW_i)‡, backfat thickness (BFT_i)‡, body-weight gain (BWG)§, rate of fat deposition (FATG)§, daily feed intake (FI_i)‖, residual feed intake (RFI_i)‖, intake time ($TIME_i$)‖, intake frequency ($FREQ_i$)‖ and intake rate ($RATE_i$)‖*

	N	Mean	s.e.
$TOTAL_{43}$ (mg/dl)	202	78.3	0.965
HDL_{43} (mg/dl)	202	31.3	0.453
LDL_{43} (mg/dl)	202	39.4	0.605
$TRIG_{43}$ (mg/dl)	202	47.7	1.33
$TOTAL_{187}$ (mg/dl)	199	122.1	1.72
HDL_{187} (mg/dl)	199	51.2	0.785
LDL_{187} (mg/dl)	199	62.1	1.11
$TRIG_{187}$ (mg/dl)	199	56.0	1.78
BW_{170} (kg)	179	105.2	0.912
BFT_{170} (mm)	178	20.7	0.307
BWG (g/day)	190	889	9.41
FATG (mm/day)	186	0.185	0.00429
$FI_{170–175}$ (kg/day)	179	3.22	0.0434
$RFI_{170–175}$ (kg)	179	0.00	0.0245
$TIME_{170–175}$ (min)	179	61.8	1.03
$FREQ_{170–175}$	179	6.47	0.285
$RATE_{170–175}$ (kg/min)	179	0.0538	0.000867

†Measured at i = an average of 43 and 187 days of age.
‡Measured at i = 170 days of age.
§Measured for the entire feed intake trial.
‖Measured between i = 170 to 175 days of age.

correlations of cholesterol and triglyceride levels between the two measurements at different ages were low and not significant: $r = 0.01$ and $P = 0.936$ for total cholesterol, $r = 0.05$ and $P = 0.517$ for HDL cholesterol, $r = 0.02$ and $P = 0.813$ for LDL cholesterol, and $r = 0.10$ and $P = 0.172$ for triglyceride plasma levels.

Table 3 presents phenotypic correlations between $TOTAL_{43}$, HDL_{43}, LDL_{43} and $TRIG_{43}$, $TOTAL_{187}$, HDL_{187}, LDL_{187}, and $TRIG_{187}$, and BW_{170}, BFT_{170}, BWG, FATG, $FI_{170–175}$, $RFI_{170–175}$, $TIME_{170–175}$, $FREQ_{170–175}$ and $RATE_{170–175}$. Animals with high levels of total cholesterol also had high levels of HDL, LDL and triglycerides. Animals with high levels of HDL also had high levels of LDL and triglycerides. Animals with high levels of LDL also had high levels of triglycerides (Table 3).

Table 3 shows a positive and highly significant phenotypic correlation between BW_{170}, BWG, BFT_{170}, FATG, $FI_{170–175}$, $RFI_{170–175}$ and $RATE_{170–175}$ and $TOTAL_{187}$, HDL_{187} and LDL_{187}. The correlation was just not significant for the relationship between $RFI_{170–175}$ and HDL_{187}.

Table 4 presents phenotypic correlations between $TOTAL_{187}$, HDL_{187} and LDL_{187} cholesterol and FI and RFI estimated for the periods between 95 and 115, 115 and 135, 135 and 155, and 155 and 175 days of age. The correlation between FI and all cholesterol measurements were significantly positive in all periods. The correlation between RFI, and $TOTAL_{187}$ and LDL_{187} was significant for the period between 155 and 175 days of age only.

Table 3 *Phenotypic correlations between total ($TOTAL_i$)†, high density lipoprotein (HDL_i)† and low-density lipoprotein (LDL_i)† cholesterol and triglyceride levels ($TRIG_i$)†, and body weight (BW_i)‡, backfat thickness (BFT_i)‡, body-weight gain (BWG)§, rate of fat deposition (FATG)§, daily feed intake (FI_i)‖, residual feed intake (RFI_i)‖, intake time ($TIME_i$)‖, intake frequency ($FREQ_i$)‖ and intake rate ($RATE_i$)‖*

	$TOTAL_{43}$	HDL_{43}	LDL_{43}	$TRIG_{43}$
$TOTAL_{43}$		0.80***	0.89***	0.48***
LDL_{43}	0.89***	0.51***		0.25***
$TRIG_{43}$	0.48***	0.27*	0.25***	
	$TOTAL_{187}$	HDL_{187}	LDL_{187}	$TRIG_{187}$
$TOTAL_{187}$		0.82***	0.90***	0.36***
LDL_{187}	0.90***	0.52***		0.19**
$TRIG_{187}$	0.36***	0.17*	0.19**	
BW_{170}	0.31***	0.29***	0.30***	-0.07
BFT_{170}	0.50***	0.48***	0.43***	0.02
BWG	0.36***	0.35***	0.33***	-0.04
FATG	0.47***	0.46***	0.40***	0.02
$FI_{170–175}$	0.43***	0.36***	0.41***	0.05
$RFI_{170–175}$	0.23**	0.14¶	0.24**	0.09
$TIME_{170–175}$	0.12	0.08	0.11	0.10
$FREQ_{170–175}$	−0.11	−0.09	−0.09	−0.07
$RATE_{170–175}$	0.22**	0.19**	0.22**	−0.04

†Measured at i = an average of 43 and 187 days of age.
‡Measured at i = 170 days of age.
§Measured for the entire feed intake trial.
‖Measured between i = 170 and 175 days of age.
¶$P < 0.10$.

Table 4 *Phenotypic correlations between total ($TOTAL_i$)†, high-density lipoprotein (HDL_i)† and low-density lipoprotein (LDL_i)† cholesterol, and daily feed intake (FI_i)‡ and residual feed intake (RFI_i)‡*

	$TOTAL_{187}$	HDL_{187}	LDL_{187}
$FI_{95–115}$	0.20**	0.17*	0.22**
$FI_{115–135}$	0.21**	0.21**	0.20**
$FI_{135–155}$	0.35***	0.35***	0.31***
$FI_{155–175}$	0.40***	0.35***	0.38***
$RFI_{95–115}$	−0.03	−0.02	0.02
$RFI_{115–135}$	0.13§	−0.09	−0.11
$RFI_{135–155}$	−0.01	0.01	−0.01
$RFI_{155–175}$	0.16*	0.08	0.18*

†Measured at i = an average of 187 days of age.
‡Measured between i = 95 and 115, 115 and 135, 135 and 155, and 155 and 175 days of age.
§$P < 0.10$.

Discussion

As the relationship between FI behaviour and cholesterol levels in humans is hard to measure accurately, the present

https://doi.org/10.1017/S1751731107000018 Published online by Cambridge University Press

study aimed at investigating the relationship between FI behaviour and cholesterol plasma levels in an animal model. Pigs have been used extensively in human nutrition research because they are similar in several key areas (Cooper *et al*., 1997; Darragh and Hodgkinson, 2000). They are monogastric, meal-eating, omnivorous mammals. The gastrointestinal anatomy, physiology and metabolism of the pig are very similar to those of the human (Moughan *et al*., 1994). Animals in the present study had *ad libitum* access to feed of a constant composition and quality, something that is hardly conceivable in human subjects.

Validation of plasma lipids profile in a pig model

Results of the present study showed that cholesterol and triglyceride plasma levels were higher at 187 days of age than at 43 days of age. This supports the observation that as animals mature, the amount of cholesterol in their tissues generally increases (Werdi Pratiwi *et al*., 2006). In our study, correlations between cholestrol and triglyceride measurements at about 43 and 187 days of age were low and not significant. In humans, the Busselton study provided a rare opportunity for longitudinal analysis of cholesterol tracking (Adams *et al*., 2005). Children were aged between 5 and 18 years when they attended a children's survey, and between 19 and 44 year at the adult surveys. The age- and survey-year-adjusted correlation coefficients ranged from 0.35 to 0.55. To our knowledge, there is no study with repeated measurements in humans from weaning to maturity.

In the present study, total cholesterol concentration was highly significantly phenotypically related with LDL and HDL cholesterol concentrations. In humans a positive relationship between total and LDL cholesterol is observed also (Lam *et al*., 1990; Sun *et al*., 2000) which suggests that total cholesterol can be used as the initial screening to identify persons who may need lipoprotein analysis (Lam *et al*., 1990). Pond *et al*. (1993) observed in pigs selected for high total plasma cholesterol concentration at 8 weeks of age a positive phenotypic correlation between total and HDL cholesterol ($r = 0.88$); LDL concentration was not measured. They suggested that genetic selection for high total plasma cholesterol levels may thus be related more to changes in HDL cholesterol concentration than to changes in the LDL cholesterol fraction. Results of the present study suggest that this may not be the case.

In the present study, LDL and HDL cholesterol plasma levels were highly significantly positively correlated. A significant relationship between HDL and LDL cholesterol levels is observed in humans also, but this is observed to be negative (e.g. Fredenrich and Bayer, 2003; Olswold and De Andrade, 2003). This observation may be related to the divergence for lipoprotein metabolism across species (Pond and Mersmann, 1996). Compared with humans, in pigs there is a low synthesis of LDL from VLDL (Birchbauer *et al*., 1992), yet transfer of HDL-cholesterol esters to LDL occurs at a high rate in the pig (Terpstra *et al*., 1993). This observation may imply also differences between pigs and humans in health risk related to cholesterol levels. The meaning of this result for cholesterol research in pigs as a model for humans warrants further research.

In the present study, triglyceride plasma levels were (highly) positively phenotypically correlated with total, HDL and LDL cholesterol plasma levels (Table 3). This contradicts the suggestion by Pond *et al*. (1993) that triglyceride levels had no relationship to total or HDL cholesterol levels, 'indicating an independent mode of inheritance'. In humans, the relationship between triglyceride and HDL levels is found to be negative (Sprecher *et al*., 1994; Dobiasova and Frohlich, 2001).

The relationship between fatness, body weight and cholesterol plasma levels in a pig model

Results of the present study indicate that cholesterol and triglyceride plasma levels measured at 187 days of age were very highly positively related with BW at 170 days of age. In mice, Dunnington *et al*. (1981) observed an increased BW of females with selection for high total cholesterol concentrations. Furthermore, selection for high BW at 56 days of age was accompanied by an increase in total plasma cholesterol (Dunnington *et al*., 1981). In a divergent selection experiment for total plasma cholesterol at 56 days of age in pigs, average BW at birth and at 4 and 8 weeks of age were higher in the high line than in the low line; BW at 164 days of age did not differ significantly between the lines (Young *et al*., 1993). In the same selection experiment, Pond *et al*. (1993) observed high phenotypic correlations between 4- and 8-week BW and total cholesterol ($r = 0.44$ and 0.46, respectively), HDL cholesterol ($r = 0.44$ and 0.46, respectively) and triglyceride levels ($r = 0.26$ and $r = 0.19$, respectively).

The present study shows a positive and highly significant correlation between cholesterol levels measured at about 187 days of age and BW gain in pigs. In the divergent selection experiment for cholesterol in pigs (Young *et al*., 1993), pigs from the high line grew 13% faster between birth and 4 weeks of age and 43% faster between 4 and 8 weeks of age, but growth rate was similar from 80 to 164 days of age. BW gain was positively correlated with total cholesterol concentration ($r = 0.46$; Pond *et al*., 1997). Little is known about the correlation between normal (non-obese) growth and cholesterol levels in humans. Mortaz *et al*. (2001) observed a higher cholesterol synthesis and lower cholesterol absorption efficiency in children who showed the greatest increase in weight centile between birth and 8–12 years of age, but only children born pre-term were considered in that study.

In the present study, the correlation between total, HDL and LDL levels at about 187 days of age, and BFT at about 170 days of age was positive and highly significant. Dunnington *et al*. (1977) observed a positive correlation between percent body fat and total plasma cholesterol in random-bred albino mice. In the divergent selection experiment in pigs, Harris *et al*. (2004) observed no differences between the high and the low cholesterol line in

https://doi.org/10.1017/S1751731107000018 Published online by Cambridge University Press

fat deposition at the first rib, 10th rib, last rib, or last lumbar vertebra. In the same experiment, Lu *et al*. (1995) observed a smaller BFT in high cholesterol line boars compared with low cholesterol line boars. In humans, people who are overweight generally have higher total and LDL cholesterol, lower HDL cholesterol and higher triglycerides (Hagan *et al*., 1983; Devroey *et al*., 2004; Wilsgaard and Arnesen, 2004).

The influence of FI behaviour on cholesterol plasma levels

In the present study, animals with higher $FI_{170–175}$ had higher cholesterol levels at 187 days of age. Feed intakes accumulated over four periods of 20 days between 95 and 175 days of age were all highly positively correlated with $TOTAL_{187}$, HDL_{187} and LDL_{187}. This may indicate a long-term relationship between cholesterol levels and FI. Also, animals with higher $RFI_{170–175}$ had higher cholesterol levels at 187 days of age; the correlation with HDL_{187} tended to be significant. However, RFI estimated for three periods of 20 days between 95 and 155 days of age was not significantly correlated with any of the cholesterol measurements. Only the correlation for the period between 155 and 175 days of age was significantly correlated with $TOTAL_{187}$ and LDL_{187}. This may indicate a short-term relationship between feed efficiency and cholesterol levels only.

RFI as defined in the present study is an estimate of the amount of feed consumed adjusted for variation in metabolic BW, growth and fatness. The results indicate that animals that consumed feed over and above their expected requirements based on their metabolic BW, growth and level of fatness, had higher cholesterol levels. In the divergent selection experiment in pigs, no relationship was observed between FI and feed efficiency between 90 and 164 days of age, and total cholesterol concentration at 8 weeks of age (Young *et al*., 1993). These results may support the fact that a relationship between feed efficiency and cholesterol levels is a short-term relationship only. No literature could be found referring to the relationship between food quantity intake (independent of food quality) or food efficiency, and cholesterol measurements in humans. As it would be practically unfeasible to measure food efficiency in humans eating food of one standard composition and quality, food efficiency in humans could alternatively be expressed in energy units.

In the present study, animals with a higher rate of FI had higher levels of cholesterol, but no relationship was observed between intake time or intake frequency, and cholesterol and triglyceride plasma levels. The effect of timing of FI on metabolism has been the subject of active investigation in humans for over 40 years (Parks and McCrory, 2005). Variation in cholesterol synthesis appears to be strongly dependent on meal timing (Cella *et al*., 1995) and meal frequency (Gwinup *et al*., 1963; Jenkins *et al*., 1989; Titan *et al*., 2001; Farshchi *et al*., 2004). The question of whether there is a health benefit from the consumption of multiple small meals will ultimately depend on how much energy is consumed, as opposed to how often or how regularly one eats (Parks and McCrory, 2005). However, the association between meal frequency and plasma cholesterol in the study of Titan *et al*. (2001) was still present after adjustment was made for body mass index, physical activity, cigarette smoking and dietary intake. In the present study, adjusting cholesterol plasma levels and intake rate for the level of FI, did decrease the phenotypic correlations considerably, to $r = 0.09$, 0.09 and 0.10 for the correlation with total, HDL and LDL cholesterol, respectively; values were no longer significant. This indicates that the positive correlation between intake rate and cholesterol measurements were mainly a result of a correlated increase in FI.

To summarise, this study showed in a pig model that animals that were heavier, fatter, growing faster, with a higher rate of fat deposition, consuming more feed, at a higher rate, and being less feed efficient, had higher levels of total, HDL and LDL cholesterol. This study did not observe any relationship between intake time nor frequency and cholesterol plasma levels. Results indicate that the relationship between FI and cholesterol levels is a long-term relationship, while the relationship between RFI and cholesterol levels is more of a short-term nature. The relationship between intake rate and cholesterol plasma levels disappeared after correction for the amount of feed consumed. Results indicate that FI independent of metabolic BW, growth and fatness, i.e. 'RFI', was positively correlated with total and LDL cholesterol plasma levels and tended to be positively correlated with HDL cholesterol plasma levels. These results add a new dimension to the influence of food on cholesterol levels, as besides the influence of food quality and obesity as a result of overeating, the results suggests that eating food over and above the maintenance and growth requirements, but independent of the level of fatness, constitutes a health risk. The mechanism behind this relationship and its implications may be further investigated.

Acknowledgements

We are grateful to the Spanish Ministry of Science and Technology (MCYT) for financing this research through the Project AGL2002-04271-C03-02 entitled 'Arquitectura genética de los components lipídicos de la carne porcina relacionados con la calidad y la salud humana'. We are also very grateful to the company Selección Batallé for providing the Duroc pigs and for making this research possible.

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https://doi.org/10.1017/S1751731107000018 Published online by Cambridge University Press