Eur J Nutr 40 : 155–160 (2001)

ORIGINAL CONTRIBUTION

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Effect of 24-h food deprivation on lipoprotein composition and oleoyl-estrone content of lean and obese Zucker rats

Summary *Background* Food deprivation induces the mobilization of fat reserves and, consequently, the transport of lipids in plasma. Zucker obese rats are grossly hyperlipidemic and do not use lipids as an efficient energy substrate. They also have lower circulating levels of acyl-estrone than expected because of their large fat stores.

Aim of the study To measure the effect of 24 h food deprivation on hyperlipidemia and acyl-estrone distribution in plasma in Zucker obese rats. *Methods* The plasma lipoprotein distribution and composition of Zucker lean (*Fa/?*) and obese (*fa/fa*) rats was determined after 24 hours of food deprivation. Lipid classes: phospholipid, free and esterified cholesterol and triacylglycerols, and protein and total (mainly acyl-) estrone were also measured in total plasma and lipoprotein fractions. *Results* Food-deprived rats showed lower triacylglycerol levels than fed rats, but obese rats maintained high lipid levels, mainly in the VLDL fraction. The ratio of total plasma free-to-esterified cholesterol was lower in fed lean rats (0.29) than in the obese (0.61); the situation improved slightly after 24-h starvation, since the corresponding ratios were 0.30 and 0.41. Acyl estrone levels changed little with 24-h food deprivation. The chylomicra + VLDL total estrone compartment was essentially unchanged in lean and obese fed and starved groups, but the HDL pool decreased with food deprivation in the obese. *Conclusion* Short-term starvation helped to enhance the differences between lean and obese Zucker rats in the handling of lipoprotein lipids, the latter showing a marked impairment in their ability to dispose of circulating lipids. The different pace of plasma lipid utilization may compound the problems of cholesterol transfer, partly explaining the dyslipemia that characterizes this animal model of obesity. The differences in acyl-estrone distribution also indicate that fat mass is preserved more effectively in obese rats even after food deprivation.

Key words Food deprivation – Obesity – Zucker fa/fa – Lipoproteins – Cholesterol – Acyl-estrone

Received: 23 April 2001
Accepted: 13 July 2001

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Introduction

Genetically obese Zucker *fa/fa* rats are dyslipemic [1] and have a limited ability to dispose of chylomicra [2]. The altered synthesis of fat by the liver [3] results in an excess of circulating lipids [4] and the massive deposition of fat stores in peripheral tissues [5]. This is associated with a limited thermogenic ability [6] and disrupted endocrine homeostasis, in which type II diabetes is often the main component [7].

In the Zucker obese rat, circulating levels of chylomicra and VLDL are higher than in lean controls [8]; hepatic production of VLDL is increased [9]. In genetically obese rats, the clearance of chylomicra is lower than in lean controls [2], and their hypertriacylglycerolemia has been attributed to the accumulation of chylomicron remnants that are not processed rapidly enough by the liver [2].

Zucker *fa/fa* rats have similar or lower [10–12] lipoprotein lipase activity in peripheral tissues, which has been offered as an explanation of both the reduced disposal of circulating fats and their gross accumulation of adipose tissue.

During and after a meal, most of the blood lipids are carried by chylomicra. The wide variation in their size and their constant shrinking due to the release of fatty acids by lipases make them difficult to analyze; their presence tends to obscure the changes occurring in other more stable lipoproteins. For this reason, they are usually omitted from analyses of plasma lipids, in which plasma is usually taken after a short period of fasting (i. e., overnight). This eliminates chylomicra from the samples, but also precludes the study of the changes in plasma lipid transport in a critical period, when the fate of dietary fat is probably determined. The handling of the chylomicron peak, just after a meal, is critical for the deposition or disposal of dietary fat, which influences the development and maintenance of obesity [13].

Our previous analysis of lipoproteins in Zucker obese rats under postprandial conditions showed that the largest differences in lipid content of the blood and lipoprotein particle dynamics were indeed observed precisely in these conditions, which emphasizes the role of chylomicra [14]. In order to gain insight into how the Zucker *fa/fa* rat handles the large amount of lipids present in the blood, we compared the lipoprotein profile and composition of Zucker *fa/fa* rats after feeding and after 24 h of food deprivation. We thus aimed at examining the effect of starvation on the dynamics of large lipoprotein particles and the lipid class distribution in the smaller lipoproteins.

Oleoyl-estrone has been postulated as a blood-carried lipostatic signal [15]; its administration to rats results in dose-dependent losses of fat, sparing body protein [16, 17]. Most of the circulating acyl-estrone is carried in the chylomicra + VLDL fractions [18], but there is evidence that the main physiologically active compartment is related to HDL [19]. In addition, obese rats maintain lower circulating acyl-estrone levels than would be expected from their fat mass [20]; this deficit, coupled with their higher sensitivity to oleoyl-estrone administration than the lean [20], strongly suggests that the oleoyl-estrone synthesis is defective in Zucker obese rats.

Here, we examined the effects of starvation on the lipoprotein distribution of plasma acyl-estrone, given that the small variations in its circulating levels induced by food deprivation correlate closely with overall body fat content [21].

Materials and methods

Two groups of female adult Zucker lean (*Fa/?*) rats weighing 224 ± 7 g, and two groups of 3-month-old obese (*fa/fa*) rats weighing 407 ± 12 g were fed and maintained in standard controlled conditions (lights on from 08:00 h to 20:00 h). One group of lean and one group of obese rats had all food removed from their cage at the beginning of a light cycle. Control groups had food pellets (energy content 14.0 MJ/kg; containing 20 g/kg of fat) continuously available, and all groups had free access to water at all times. Food consumption under basal conditions in lean rats was 16.0 ± 0.5 g/day and 21.6 ± 0.5 g/day in the obese.

All rats were decapitated at the beginning of the next light cycle, i. e., after 0 or 24 h of food deprivation. Their blood was collected in dry EDTA-Na-treated beakers. Plasma was immediately obtained by centrifugation and then used for the separation of plasma lipoproteins. Frozen aliquots were used for the analysis of total plasma protein and lipid components.

Lipoproteins in fresh plasma were fractionated by differential ultracentrifugation in known density media obtained through the addition of KBr [22]. Five main fractions were obtained: chylomicra (CM) [density lower than 1.000 g/mL], very low density lipoproteins (VLDL) [1.000 g/mL–1.006 g/mL], low density lipoproteins (LDL) [1.006 g/mL–1.063 g/mL], high density lipoproteins (HDL) [1.063 g/mL–1.210 g/mL] and the lipoprotein-depleted plasma (LPdp) [greater than 1.210 g/mL].

Whole plasma, lipoprotein fractions and LPdp were used for the analysis of protein and lipid content. Total protein was estimated with the Bradford method [23]. Total and free cholesterol were measured in saline with a high-performance cholesterol kit (139 050 Roche, Mannheim, Germany). Triacylglycerol concentrations were measured with a commercial kit (COD11528 Biosystems, Barcelona, Spain) and the results were corrected for free glycerol. Phospholipid was quantified by a choline-based method (PLMPR2691844 Roche).

The levels of total (essentially acyl-) estrone were determined in the lipid extracts of all fractions by alkaline hydrolysis followed by specific radioimmunoassay [24].

The significance of the differences between groups was established using a two-way anova program.

Results

Table 1 shows the concentration of protein, lipid classes and acyl-estrone in the plasma of lean and obese rats after 24 hours of food deprivation and in fed controls. Fig. 1 shows the composition of whole plasma, chylomicra, VLDL, LDL and HDL, as well as lipoprotein-depleted plasma (LPdp) of fed and 24-h starved lean and obese rats. Table 2 presents the corresponding protein values.

In lean rats, 24-h starvation resulted in lower plasma triacylglycerol levels, with unchanged protein, phospholipid and cholesterol concentrations. When compared

Table 1 Plasma protein and lipid content of fed and 24-h starved lean and obese Zucker rats

		Lean		Obese	
Fraction	Units	Fed	24-h starved	Fed	24-h starved
Protein	g/L	63.9±2.8	61.9±0.3	65.8±2.8	62.8±6.4
Triacylglycerols	mM	1.14±0.13	0.56±0.07	8.34±1.00	4.60±0.20
Phospholipids	mM	2.13±0.11	2.22±0.03	4.92±0.28	3.65±0.13
Free cholesterol	mM	0.40±0.03	0.49±0.03	1.21±0.15	0.99±0.07
Cholesterol esters	mM	1.40±0.09	1.61±0.06	1.99±0.14	2.40±0.07

The data are the mean ± sem of 6–8 animals. Statistical analysis of the differences between groups (two-way anova, only significant, $p<0.05$ values are given): criteria "obesity", triacylglycerols p=0.0000, phospholipids p=0.0000, free cholesterol p=0.0000, cholesterol esters p=0.0001; criteria "starvation", triacylglycerols p=0.0061, cholesterol esters p=0.0049.

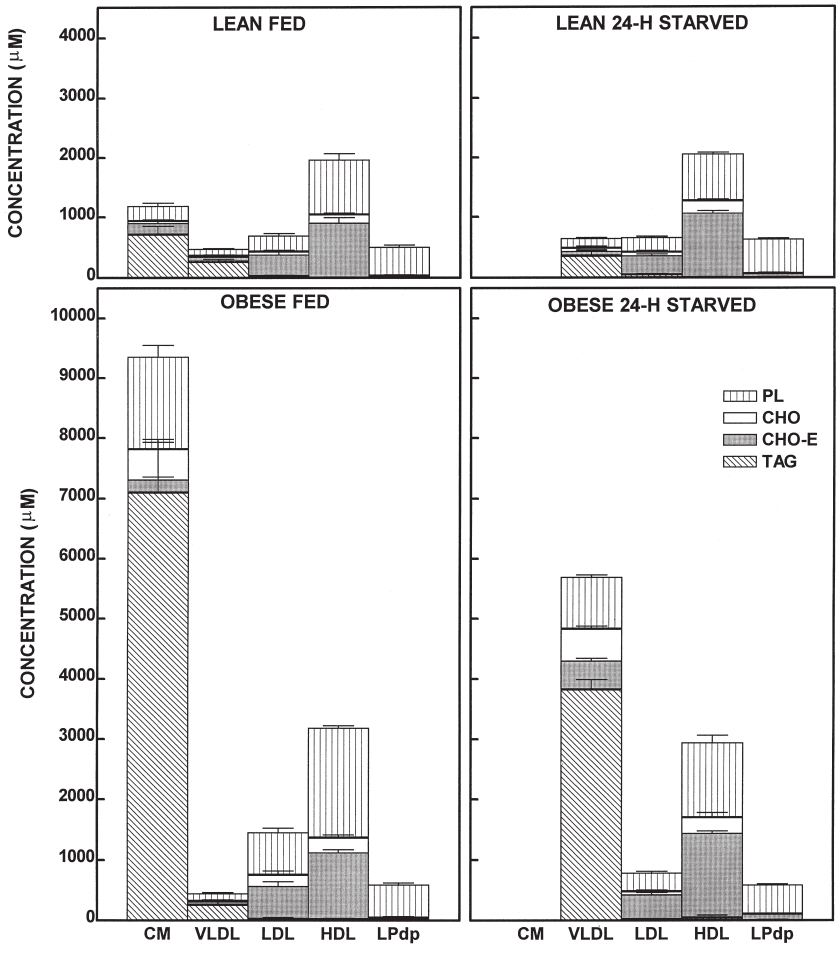

Fig. 1 Distribution of lipid classes in lipoprotein fractions of fed and 24-h starved lean and obese Zucker rats. The data are the mean ± sem of 6–8 different animals. *PL* phospholipid; *CHO* free cholesterol; *CHO-E* cholesterol esters; *TAG* triacylglycerol. Statistical analysis of the differences between groups (two-way anova, only significant, $p<0.05$ values are given): 1-Triacylglycerols, criteria "obesity", CM+VLDL p=0.0027, HDL p=0.0499, criterion "starvation", CM+VLDL p=0.000. 2-Phospholipids, criteria "obesity", CM+VLDL p=0.0019, LDL p=0.0001, HDL p=0.0000, criteria "starvation", CM+VLDL p=0.000, LDL p=0.0082, HDL p=0.0038. 3-Free cholesterol, criteria "obesity", CM+VLDL p=0.0000, HDL p=0.0077, criterion "starvation", LPdp p=0.0421. 4-Cholesterol esters, criteria "obesity", CM+VLDL p=0.0395, LDL p=0.0201, HDL p=0.0017, criterion "starvation", HDL p=0.0265.

with the lean, fed obese rats showed similar protein content, but had much higher levels of triacylglycerols, phospholipids and total and free cholesterol. After 24-h food deprivation, the differences versus lean animals were maintained for all lipid classes; in addition, there were significant decreases in triacylglycerols, and phospholipids.

The chylomicra fraction was not detectable in starved animals. However, in the fed state, obese rats presented a higher mass of all lipid classes in this fraction than the lean (except for esterified cholesterol). The VLDL fraction showed marked differences between lean and obese rats and following food deprivation: obese rats had higher levels of lipids – but not of protein – after starvation; in the fed state, these animals had lower protein and cholesterol ester levels than the lean. In lean rats, starvation induced an increase in VLDL phospholipids and free cholesterol. In the obese, triacylglycerol,

Table 2 Protein content of the lipoprotein fractions of fed and 24-h starved lean and obese Zucker rats

		Lean		Obese	
Fraction	Units	Fed	24-h starved	Fed	24-h starved
CM	mg/L	629±29	–	823±56	–
VLDL	mg/L	39±3	34±3	28±2	39±7
LDL	mg/L	89±11	185±38	145±37	250±19
HDL	mg/L	631±84	292±50	986±47	558±26
LPdp	g/L	56.6±1.8	61.2±0.2	54.5±2.4	61.9±6.4

The data are the mean ± sem of 6–8 animals. Statistical analysis of the differences between groups (two-way anova, only significant, $p<0.05$ values are given): criterion "obesity", HDL p=0.0003; criteria "starvation", CM+VLDL p=0.0000, LDL p=0.0012, HDL p=0.0000.

phospholipid and cholesterol levels increased after food deprivation.

The levels of phospholipid and free cholesterol in the LDLs of obese rats were higher than those of the lean in the fed state; starvation increased triacyglycerol and protein levels in lean rats, but resulted in lower phospholipid and increased protein levels in the obese.

In the fed state, the HDLs of obese rats contained higher levels of protein, triacylglycerols, phospholipids and free cholesterol. Starvation, decreased HDL protein, but increased free cholesterol levels in the lean. In contrast, starvation decreased protein and phospholipids and increased cholesterol esters in the obese; when compared with starved lean rats, the obese maintained high protein, phospholipid and cholesterol ester levels.

Lipoprotein-depleted plasma composition was more uniform. Food deprivation caused limited increases in the cholesterol-ester content of obese rats

Acyl-estrone levels and distribution changed little with starvation: 117 ± 39 nM in lean and 141 ± 20 nM in obese rats in the fed state, and 129 ± 26 nM and 145 ± 26 nM, respectively, after 24-h starvation. The distribution of this hormone in lipoprotein fractions is shown in Fig. 2. The pattern was practically identical in lean and obese fed rats, with deep changes induced by starvation: disappearance of chylomicra acyl-estrone and – only in the obese – a decrease in the HDL fraction content of estrone.

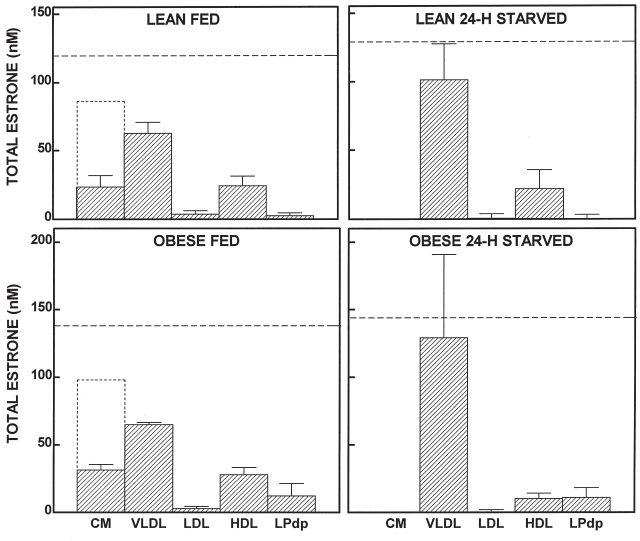

Fig. 2 Distribution of total estrone in lipoprotein fractions of fed and 24-h starved lean and obese Zucker rats. The data are the mean ± sem of 6–8 animals. The dashed line indicates the mean total estrone plasma levels in the corresponding groups. The dot line column reflects the sum of chylomicra and VLDL estrone in the control groups.

Discussion

Zucker obese rats showed higher plasma lipid levels than their lean counterparts in both fed and starved conditions [20]. This was a consequence of the huge mass of lipids in the chylomicra + VLDL fraction [14], and high circulating cholesterol [1, 25]. Short-term starvation slightly reduced this mass of lipid – mainly triacylglycerols –, but in food-deprived obese rats the lipid remaining in this fraction was many times larger than that of controls.

The maintenance of a large circulating triacylglycerol pool in Zucker fa/fa rats has been postulated to be a consequence of their limited ability to dispose of chylomicra [2]. This inability has been related to lower peripheral lipoprotein lipase activity [11, 12], but other reports show that lipoprotein lipase activity is not affected in this animal model of obesity [10]. The alterations due to size and specific apoprotein deficits observed in the lipoproteins of Zucker *fa/fa* rat may play a more relevant role in their loss of lipid transfer function [14].

Transfer of cholesterol to tissues depends largely on the proportion of free to esterified cholesterol carried by lipoproteins [26]. In obese rats, the insufficient capacity to process circulating triacylglycerols is compounded by their altered ability to form cholesteryl-esters from free cholesterol, and to transport this steroid between tissues [27]. Consequently, the total plasma free-to-esterified cholesterol ratio was lower in fed lean rats (0.29) than in the obese (0.61); the situation improved slightly after 24-h starvation, since the corresponding ratios were 0.30 and 0.41. The changes in all the fractions studied (except HDL, in which there was a higher degree of uniformity) followed the same pattern. In the chylomicra + VLDL fraction, the differences in the fed state between lean and obese rats were more marked, with free-to-esterified cholesterol ratios of 0.37 and 2.36, respectively; after 24-h of food deprivation, the ratios of both groups coincided: 1.2. The differences described point to a marked difficulty in the processing of lipoprotein cholesterol, together with lower esterification rates in the obese animals [14, 27]. This is consistent with the larger

lipoprotein particle size in Zucker *fa/fa* rats [14], since free cholesterol remains in the lipoprotein surface and its esters sink to the core.

The uniformity observed in the pattern of distribution and levels of acyl-estrone in lipoprotein fractions agrees with previous data on distribution in lean rats [18], and the low circulating acyl-estrone found in obese rats when their high fat mass is taken into account [20]. Obese humans show lower plasma acyl-estrone than expected [28], since they do not follow the direct relationship found between fat mass and circulating acyl-estrone levels in normal weight individuals [29]. Starvation did not significantly affect the levels of acyl-estrone in plasma [21], but its distribution was modified: the acyl-estrone distributed between chylomicra and VLDL in the fed stare was now concentrated in the VLDL fraction, irrespective of its lipid content. Indeed, the amount of acyl-estrone carried by VLDL and chylomicra was essentially the same in all four groups studied, pointing to a highly preserved pool irrespective of the changes occurring in the lipid, mass and composition of these large lipoproteins.

Starved obese rats also showed a decrease in the acyl-estrone content of the HDL fraction. This change may have a deeper significance in protecting body fat, since it has been postulated that the oleoyl-estrone in the HDL compartment has a more important physiological role in the control of fat stores [19]. A decrease in the oleoyl-estrone levels in the HDL fraction may weaken this ponderostat signal, thus decreasing the activity/effectiveness of the lipolytic signals and potentiating fat preservation and accumulation.

Short-term starvation helped to enhance the differences between lean and obese Zucker rats in the handling of lipoprotein lipids, the latter showing a marked impairment in their ability to dispose of circulating lipids. The different pace of plasma lipid utilization may compound the problems of cholesterol transfer, partly explaining the dyslipemia that characterizes this animal model of obesity. The differences in acyl-estrone distribution also indicate that fat mass is preserved more effectively in obese rats even after food deprivation.

Acknowledgments This study was financed by grants 2FD97–0233 and AGL2000–0780 of the Government of Spain. Thanks are given to Robin Rycroft from the Language Advisory Service at the University of Barcelona for corrections to the text.

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