Increased postprandial fatty acid trapping in subcutaneous adipose tissue in obese women

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Abstract The objective of this study was to test the hypothesis that increased fatty acid trapping by subcutaneous adipose tissue might contribute to the development and/or maintenance of obesity. To do so, venoarterial (V-A) gradients across subcutaneous adipose tissue for triglycerides, glycerol, nonesterified fatty acid (NEFA), and acylation-stimulating protein (ASP) were determined in eight lean females [body mass index (BMI), 22.2 ± 0.6] and eight obese females (BMI, 34.4 ± 3.4). Plasma insulin was also measured at intervals throughout this period. Fasting plasma triglyceride was significantly higher in the obese group and postprandial triglyceride was also significantly delayed. In contrast, both triglyceride clearance and fatty acid uptake by subcutaneous adipose tissue were significantly greater in the obese group compared with the lean group. Fasting insulin did not differ between the groups, but postprandial insulin values were significantly higher in the obese group. The pattern of ASP release from subcutaneous adipose tissue also appeared to differ in that it was significantly greater in the early postprandial period (0–90 min) in the obese group versus the lean group and this correlated with greater triglyceride clearance during this period. Moreover, there were strong, positive correlations between BMI and the V-A gradient for fasting ASP, the 0- to 90-min area under the curve (AUC) for ASP V-A gradient fasting insulin, and the 0- to 90-min AUC for fatty acid incorporation into adipose tissue. Taken together, these data demonstrate that fatty acid trapping by adipose tissue can be increased even when overall plasma triglyceride clearance is delayed. The postprandial pattern of insulin, in particular, was altered in the obese, although it is certainly possible that differences in ASP release or response could also contribute to increased fatty acid trapping in the obese. The data, therefore, suggest that increased fatty acid trapping by adipose tissue may be a feature of some forms of obesity.—Kalant, D., S. Phélis, B. A. Fielding, K. N. Frayn, K. Cianflone, and A. D. Sniderman. **Increased postprandial fatty acid trapping in subcutaneous adipose tissue in obese women. *J. Lipid Res.* 2000. 41: 1963–1968.**

Supplementary key words obesity • triglyceride clearance • insulin • acylation-stimulating protein • C3adesArg

It remains as unclear as ever whether any substantial proportion of those who become obese are metabolically programmed to do so. In particular, much remains to be learned in what ways, if at all, the obese differ from the nonobese with respect to the uptake and release of fatty acids from adipose tissue. In the postprandial period, dietary fatty acids are transported as chylomicron triglycerides (TG) and distributed among adipose tissue, muscle, and the liver. There is, necessarily, an inverse relationship between fatty acid trapping by adipose tissue and the delivery of fatty acids to the other two sites (1). In particular, to the extent fatty acid trapping by adipose tissue is increased, delivery of fatty acids to the liver will be reduced and apolipoprotein B (apoB) secretion will be reduced as well. Therefore, in general, effective fatty acid trapping by adipose tissue is associated with normal plasma apoB.

Until recently, attention has focused on insulin as the sole regulator of fatty acid trapping by adipose tissue. Moreover, there is a widely held view that obesity is associated with insulin resistance, although whether this applies to adipocytes as well as to glucose removal by skeletal muscle is not really clear (2–4). But insulin is not the only peptide that modulates adipose tissue fatty acid balance. Acylation-stimulating protein (ASP) is a 76-amino acid peptide that is the product of the interaction of three proteins secreted by adipose tissue: C3, adipsin, and factor B (5). In the initial reaction, a 77-amino acid peptide, C3a, is produced, after which the carboxy-terminal arginine is removed by carboxypeptidase B to produce C3adesArg or ASP.

ASP stimulates adipocyte TG synthesis by increasing specific membrane transport of glucose and by increasing the activity of diacylglycerol acyltransferase, the en-

Abbreviations: ANOVA, analysis of variance; apoB, apolipoprotein B; ASP, acylation-stimulating protein; AUC, area under the curve; A-V, arteriovenous; BMI, body mass index; FIAT, fatty acid incorporation into adipose tissue; LPL, lipoprotein lipase; NEFA, nonesterified fatty acid; TG, triglyceride; V-A, venoarterial; VLDL, very low density lipoprotein.

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zyme that drives the last step in the synthesis of a TG molecule (6). ASP also reduces fatty acid release from human adipocytes by reducing hormone-sensitive lipase activity and by increasing re-esterification (7). Both effects, therefore, lead to greater entrapment of fatty acids in adipose tissue. Of importance, the effects of ASP are independent of, but additive to, those of insulin (8).

Our initial study, which examined the in vivo release of ASP from normal human subcutaneous adipose tisssue, demonstrated that *i*) ASP was released after an oral fat load, *ii*) the release of ASP increased substantially in the second half of this period, and *iii*) TG clearance and fatty acid uptake paralleled the changes in ASP release (9). Plasma insulin was also increased over this period and therefore both insulin and ASP could have contributed to the increase in fatty acid trapping by subcutaneous adipocytes that was evident in the postprandial period. Both insulin and ASP are increased in obesity and accordingly it seemed possible that obesity could be associated with increased fatty acid trapping. On the basis of the hypothesis outlined above, we reasoned that obese subjects with normal plasma apoB might manifest increased fatty acid trapping by adipose tissue. Therefore, the present study was designed to examine the effectiveness of postprandial fatty acid trapping in normal and obese females with normal plasma apoB.

MATERIALS AND METHODS

Subjects

Sixteen female subjects were studied. Some data from some of the subjects have been included in other studies (10, 11). The subjects were divided into two groups: lean and obese, with a body mass index (BMI) cutoff of 25 kg/m^2 for the lean (12). Their ages, BMI, and fasting lipid levels are shown in **Table 1**. All subjects fasted and drank only water for at least 12 h before the study. All studies were undertaken in a temperature-controlled room (23°C) and none of the subjects was taking any medication known to affect lipoprotein metabolism. All studies were approved by the Central Oxford Research Ethics Committee, and subjects gave their informed consent.

TABLE 1. Fasting arterial values for lean and obese women

	Lean (n = 8)	Obese (n = 8)	*P*
Age (years)	42.0 ± 6.0	53.0 ± 3.0	NS
BMI	22.2 ± 0.6	34.4 ± 3.4	0.0025
ApoB (mg/dl)	62.7 ± 4.6	86.0 ± 5.6	0.005
Cholesterol (mM)	4.4 ± 0.3	5.3 ± 0.4	0.05
HDL cholesterol (mM)	1.3 ± 0.1	1.0 ± 0.1	NS
ASP (nM)	58.1 ± 10.3	47.7 ± 6.9	NS
Insulin	33.1 ± 2.1	43.4 ± 7.6	NS
TG (μM)	885.0 ± 119.0	1,842.0 ± 373.0	0.025
NEFA (μM)	717.0 ± 78.0	858.0 ± 26.0	NS
Glycerol (μM)	71.7 ± 10.1	104.7 ± 7.4	0.01

All values were measured in arterial plasma except for glycerol, which was measured in whole blood. Values are reported as the mean ± standard error of the mean. Means were compared by unpaired *t*-test and the *P* values are indicated.

Experimental design

Venoarterial (V-A) studies were conducted as previously described (13). In brief, a cannula was inserted retrogradely into a hand vein and the hand was warmed in a box at 60–70°C so that arterialized blood samples could be obtained. A 10-cm, 22-gauge catheter was then introduced over a guide wire into one of the superficial veins on the anterior abdominal wall and threaded toward the groin, so that its tip lay just superior to the inguinal ligament. Samples from this cannula represent the venous effluent from the subcutaneous abdominal adipose tissue, uncontaminated by muscle drainage and with only a minor contribution from skin. Both catheters were kept patent with saline and heparin was not administered.

The subjects rested for at least 30 min after insertion of the catheters and before any samples were taken. They then ate a mixed meal containing 60 g of fat, 85 g of carbohydrate, and 13 g of protein and V-A blood samples were taken simultaneously at times 0, 30, 60, and 90 min as well as 2, 3, 4, 5, and 6 h after eating. The samples were centrifuged and plasma stored at −70°C.

Analyses

Plasma TG were measured enzymatically on an IL Monarch centrifugal analyzer (Instrumentation Laboratory, Warrington, Chesire, UK) with correction for free glycerol (14). Plasma total cholesterol was measured with a commercial enzymatic colorimetric method (cholesterol 50 kit; Sigma, Poole, UK). Plasma nonesterified fatty acid (NEFA) was measured by an enzymatic method (Wako NEFA kit; Alpha Laboratories, Eastleigh, UK). Total apoB was measured by a competitive enzyme-linked immunosorbent assay (ELISA), using rabbit anti-human apoB antibody (in house), a commercial standard (837237; Boehringer Mannheim, Laval, Quebec, Canada), and controls (Precipath 1285874 and Precinorm 781827; Boehringer Mannheim) as previously described (15). Plasma insulin was measured by a double-antibody radioimmunoassay kit (Pharmacia, Milton Keynes, UK). Glycerol was measured in whole blood (16). Human ASP was assayed as previously described (9). The intra-assay variability was 4% whereas the interassay variability was 8%. Fatty acid incorporation into adipose tissue (FIAT) was calculated as previously described (9).

Statistics

All results are presented as means ± standard error of the mean. Statistical differences for the data were calculated by repeated measures analysis of variance (ANOVA). The area under the curve (AUC) was calculated by a trapezoidal technique. The groups were compared with an unpaired *t*-test, unless the data were not normally distributed, in which case a Mann-Whitney test was applied. V-A differences were calculated for each subject at each time point individually, and the values were averaged. Arteriovenous (A-V) differences are reported for TG so that the results are positive. Correlations were analyzed by Pearson product moment correlation by computer, using the SigmaStat program (Jandel Scientific, San Rafael, CA).

RESULTS

The characteristics of the two groups of study subjects are shown in Table 1. The obese subjects were slightly older than the lean subjects, but the differences were not significant (Table 1). The BMI was by definition higher in the obese than in the lean. A number of lipid parameters such as apoB, cholesterol, high density lipoprotein choles-

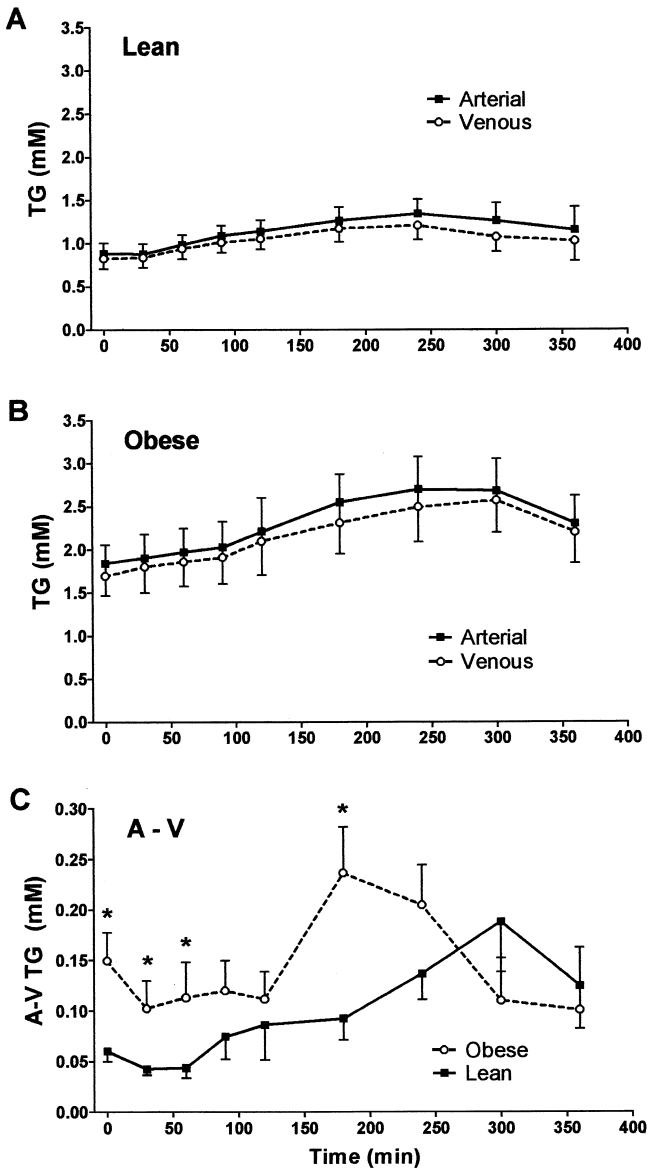

Fig. 1. Arterial, venous, and A-V plasma TG: (A) Arterial and venous TG in the lean group. (B) Arterial and venous TG in the obese. By Student's *t*-test the areas under the arterial curves are significantly different between lean and obese groups ($P = 0.023$). (C) A-V gradients across the adipose tissue bed in lean and obese groups. By ANOVA the groups are not different, but by Student's *t*-test the area under the overweight curve from 0 to 90 min is significantly greater than that for the lean ($P = 0.013$). Individual time points that are different between lean and obese groups by Student's *t*-test are indicated by an asterisk.

terol, and glycerol were significantly higher in the obese group but all mean values remained within the normal range. Surprisingly, ASP values were not significantly different between the two groups, principally because they were higher than usual in the lean individuals (17). No explanation is obvious for this difference but it applied to both the arterial and venous samples. Similarly, fasting insulin levels did not differ significantly between the two groups, although significantly higher values are more typical in the obese (18).

The arterial, venous, and A-V differences across the subcutaneous adipose tissue bed for plasma TG for both groups are shown in **Fig. 1**. Plasma TG were significantly higher in the obese compared with the lean group (Table 1). The arterial TG AUC was also significantly greater in the obese (Fig. 1B) compared with the lean (Fig. 1A) (849 ± 190 vs. 412 ± 56 mM, $P = 0.023$). As observed previously, and as would be expected, the A-V gradient for TG, that is, the decrease in TG concentration across the subcutaneous abdominal adipose tissue, became more pronounced in both groups over the postprandial period (Fig. 1C). Indeed, at all except the last two time points, the absolute TG gradient was greater in the obese than the lean, although the differences were significant only at four points. Although the overall AUC for A-V TG gradient did not differ between the two groups (53.4 ± 9.7 vs. 37.7 ± 8.2 mM), the AUC from 0 to 90 min was significantly greater in the obese compared with the lean (10.5 ± 2.3 vs. 4.4 ± 0.8 mM, $P = 0.013$).

These results are of interest when compared with ASP release by subcutaneous adipose tissue (**Fig. 2**). The lean subjects demonstrated increased release of ASP after the initial postprandial period, just as has been observed previously (9). However, the variance was sufficiently large that, as opposed to our earlier study (9), there was no significant difference between ASP release in the first and second halves of the postprandial period. There was, however, no trend for the release of ASP to increase over time in the obese group, being already maximal at the onset. Moreover, the AUC for ASP release expressed as V-A difference was significantly greater in the 0- to 90-min period in the obese compared with the lean group (2,084 ± 879 vs. 215 ± 242 mM). Thus the obese group evidenced both greater generation of ASP and greater plasma TG hydrolysis across the subcutaneous adipose tissue bed than the lean in the early postprandial period.

The V-A gradients for glycerol in both groups are shown in **Fig. 3**. The AUC gradient was again significantly greater in the obese compared with the lean group (58.3 ± 7.4 vs. 39.1 ± 7.9 mM $P = 0.033$).

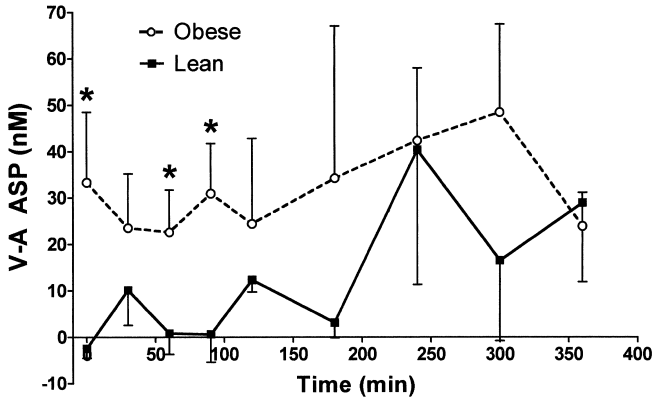

Fig. 2. V-A gradients for ASP: The obese are not different from the lean by ANOVA, but the area under the obese curve from 0 to 90 min is significantly greater than for the lean ($P = 0.030$). Individual points that are different between the two groups are indicated by an asterisk.

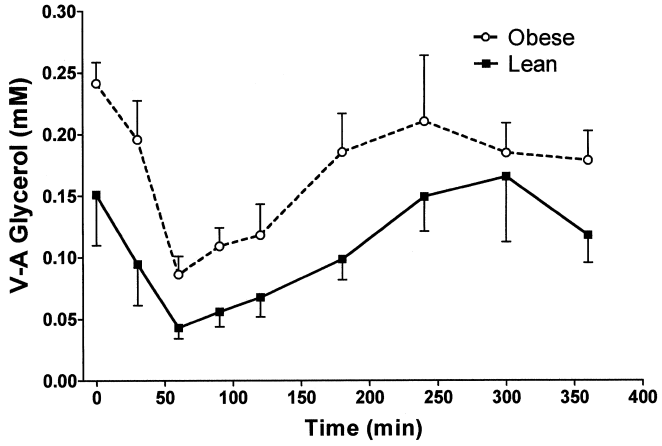

Fig. 3. V-A gradients for blood glycerol: The curve for the obese is significantly greater than for the lean, both by ANOVA (P = 0.018) and by AUC from 0 to 360 min (P = 0.033).

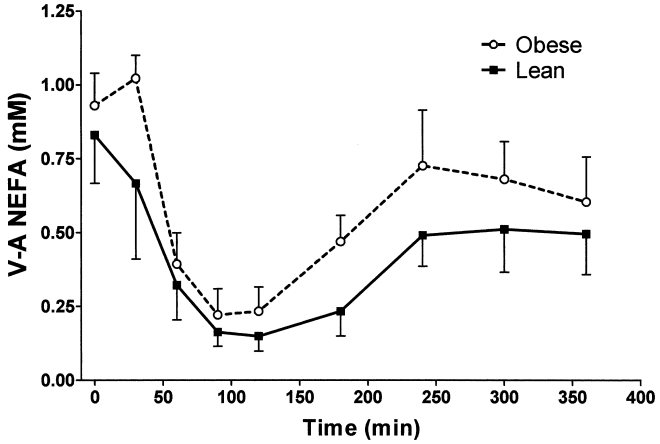

Fig. 5. V-A gradients for NEFA: V-A gradients for NEFA are not different between the lean and obese, either by ANOVA or by AUC.

There were also major differences in the insulin response between the groups during this period (**Fig. 4**). Notwithstanding that fasting levels were indistinguishable, plasma insulin concentrations increased more substantially in the obese compared with the lean in the first part of the postprandial period (Fig. 4) (P = 0.003, 0- to 90-min AUC) and these differences were so pronounced as to make the total plasma insulin significantly different by ANOVA for the two groups (P = 0.035).

As shown in **Fig. 5**, there was no overall difference between the groups in terms of the V-A gradient for NEFA. The V-A gradients for NEFA dropped sharply by 60 min into the postprandial period, and in the obese group this was achieved in the presence of higher insulin levels in plasma and greater ASP release by adipocytes. As shown in **Fig. 6**, calculated FIAT was significantly greater by ANOVA in the obese than in the lean group, pointing to greater fatty acid trapping by adipose tissue in the obese than in the lean.

To examine the relation of obesity to the metabolism of subcutaneous adipose tissue more closely, Pearson correlations were calculated. BMI was significantly related to a number of parameters. These include the V-A gradient for fasting ASP (r = 0.751, $P < 0.001$); 0- to 90-min AUC for ASP V-A gradient (r = 0.729, $P < 0.001$); arterial fasting insulin (r = 0.683, $P < 0.007$); A-V gradient for fasting TG (r = 0.819, $P < 0.001$); 0- to 90-min AUC for TG A-V gradient (r = 0.729, $P < 0.001$); postprandial drop in NEFA calculated as basal area minus 0- to 360-min AUC (r = 0.679, $P < 0.004$); 0- to 90-min AUC for glycerol V-A gradient (r = 0.752, $P < 0.001$); 0- to 90-min AUC for FIAT (r = 0.624, $P < 0.01$).

DISCUSSION

The objectives of this study were to examine whether fatty acid trapping was amplified in subcutaneous adipose tissue in obesity, and if this was the case, whether there was also evidence of increased activity of insulin and/or the

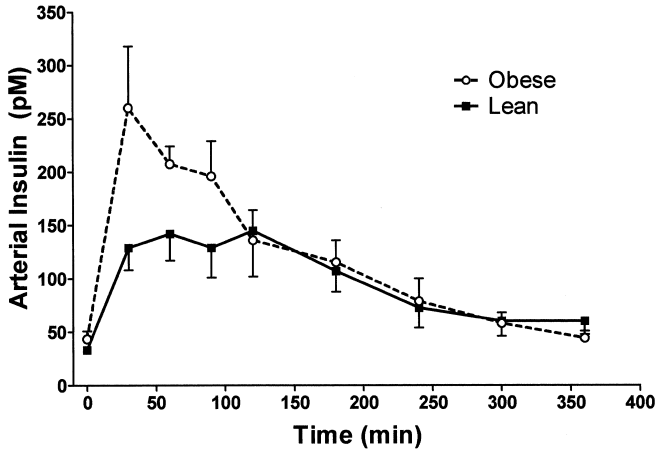

Fig. 4. Arterial insulin rises significantly higher in the obese compared with the lean, measured by AUC from 0 to 90 min (P = 0.001) as well as by ANOVA (P = 0.035).

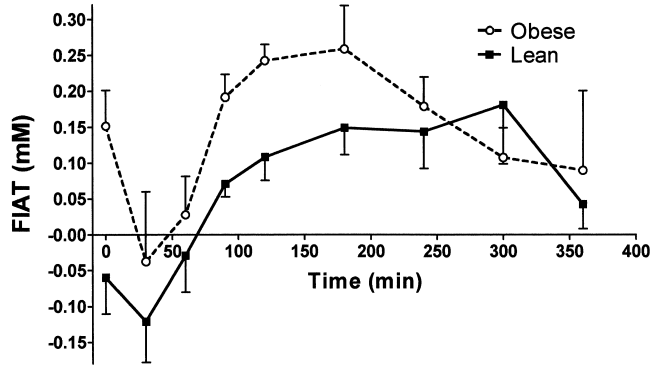

Fig. 6. FIAT was calculated by the following equation: FIAT = [3 × glycerol(V-A)] − [NEFA(V-A) × (1 − hematocrit)]. FIAT is significantly greater in the obese than in the lean as measured by ANOVA (P = 0.029).

ASP pathway. Our two prinicipal indices of fatty acid trapping, the V-A TG gradient across the subcutaneous adipose tissue bed and fatty acid incorporation into it, were concordant and greater in the obese than in the lean subjects. In contrast to previous reports, fasting plasma insulin was not higher in the obese compared with the lean group (18). However, it did rise sharply higher in the early postprandial period in the obese subjects and this difference could certainly be responsible in whole or in part for the increased fatty acid trapping in the obese subjects.

With regard to ASP, in the lean females, ASP release by subcutaneous adipose tissue appeared to accelerate at about the midpoint of the postprandial period. The differences were not significant because of the large variance but the trend is in accord with our previous report (9). In contrast, in the obese females, ASP release did not vary over the postprandial period, already being close to maximal at the onset and during this period greater than in the lean. Moreover, there were strong positive and biologically consistent correlations between BMI and ASP release, increase in plasma insulin, and all the indices of fatty acid trapping by adipose tissue.

The most straightforward interpretation of the data is that excessive fatty acid trapping by adipose tissue, probably mediated by insulin and/or ASP, is occurring in this group of obese females. Given that the differences were most pronounced in the early postprandial period, the possibility that greater transfer of TG from VLDL into adipocytes is involved must be seriously considered.

There are anomalies in the data that should not be overlooked. Many reports document that fasting insulin levels are increased, and that was not observed in this study (18). In addition, plasma ASP levels were higher in the lean group than previously observed and we have no explanation for this finding. On the other hand, ASP levels in the obese were similar to those previously recorded and therefore the findings tend to minimize any anticipated differences in ASP release between the two groups.

One observation we believe is important is that, even though fatty acid trapping was increased, there was no evidence that the overall rate of postprandial TG clearance from plasma was accelerated in the obese group. Indeed, the greater AUC for the arterial TG suggests that it is, in fact, delayed. This discordance is of interest. In the first instance, it must be recognized that only a portion of exogenous TG is directly deposited in adipose tissue during the postprandial period, the majority going either to muscle or the liver (19). There may not be, therefore, a precise correlation between the overall rate of TG clearance from plasma and the effectiveness of fatty acid trapping by adipose tissue. Second, obesity will increase the net capillary surface in adipose tissue over which TG-rich lipoproteins can interact with lipoprotein lipase (LPL), a consequence that would tend to increase the likelihood of dietary fatty acids entering adipose tissue.

More critical to appreciate is the stepwise nature of the clearance of TG-rich lipoproteins from plasma. Before a chylomicron can be hydrolyzed, it must first bind to LPL. Our in vitro studies have shown that chylomicrons have, on average, 10 times the affinity of very low density lipoprotein (VLDL) for LPL (20). On the other hand, there are approximately 10 times more VLDL particles than chylomicron particles even at the peak of postprandial TG levels. Therefore, even modest elevations in VLDL particle number, such as those observed in the overweight group, will delay chylomicron clearance by increasing the average time necessary for a chylomicron to bind to LPL. However, the effectiveness of fatty acid trapping by adipocytes is not determined by the length of time required for a chylomicron particle to find unoccupied LPL. Rather, it is a function of what happens once the particle is bound and its hydrolysis begins and fatty acid uptake by adipocytes is underway.

The evidence is decidedly mixed as to whether the obese are metabolically predestined to be so, with several reports for and against this hypothesis. A lower metabolic rate has been reported in postobese individuals compared with those who were never obese (21–23). It has also been reported that food intake must be reduced by 25% from that predicted in order for the postobese to remain at a normal weight (24). Moreover, a significant tendency to reduced oxidation of fat has also been reported (25, 26). On the other hand, there is a substantial body of evidence that fails to document any significant relevant metabolic difference between those who were obese and those who have not been (27–31).

However, Binnert et al. (32), using a stable isotope-labeled oleate, showed that a smaller portion of dietary fatty acids was oxidized and a greater portion stored in obese compared with nonobese subjects. By contrast, there was no difference found in the metabolic disposition of medium-chain TG, which are not transported in plasma in chylomicrons. This suggests that the differences observed with respect to the dietary fatty acids do relate to differences in the handling of chylomicrons by peripheral tissues. Our most recent data, obtained using methods similar to those of Binnert et al., also bear on these issues. The plasma clearance of exogenous fatty acids in women who had undergone successful gastric reduction surgery as therapy for morbid obesity was compared with that of another group of women who had never been obese. Clearance of exogenous fatty acids was much more rapid in the previously obese. The fact that oxidation of fatty acids was not higher suggests greater fatty acid trapping by peripheral tissues (unpublished observations).

In summary, the present data demonstrate that subcutaneous adipose tissue in obese females in vivo clears TG and stores the fatty acids derived from them more effectively than does adipose tissue in lean females. Moreover, the differences can be related to greater postprandial increases in plasma insulin and possibly to earlier activation of the ASP pathway in the obese subjects.

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REFERENCES

1. Sniderman, A. D., K. Cianflone, P. Arner, L. Summers, and K. Frayn. 1998. The adipocyte, fatty acid trapping, and atherogenesis. *Arterioscler. Thromb. Vasc. Biol.* **18:** 147–151.
2. Rabinowitz, D. 1970. Some endocrine and metabolic aspects of obesity. *Annu. Rev. Med.* **21:** 241–258.
3. Howard, B. V., I. Klimes, B. Vasquez, D. Brady, M. Nagulesparan, and R. H. Unger. 1984. The antilipolytic action of insulin in obese subjects with resistance to its glucoregulatory action. *J. Clin. Endocrinol. Metab.* **58:** 544–548.
4. Pei, D., Y. D. Chen, C. B. Hollenbeck, R. Bhargava, and G. M. Reaven. 1995. Relationship between insulin-mediated glucose disposal by muscle and adipose tissue lipolysis in healthy volunteers. *J. Clin. Endocrinol. Metab.* **80:** 3368–3372.
5. Baldo, A., A. D. Sniderman, S. St-Luce, R. K. Avramoglu, M. Maslowska, B. Hoang, J. C. Monge, A. Bell, S. Mulay, and K. Cianflone. 1993. The adipsin-acylation stimulating protein system and regulation of intracellular triglyceride synthesis. *J. Clin. Invest.* **92:** 1543–1547.
6. Germinario, R., A. D. Sniderman, S. Manuel, S. Pratt, A. Baldo, and K. Cianflone. 1993. Coordinate regulation of triacylglycerol synthesis and glucose transport by acylation stimulating protein. *Metabolism.* **42:** 574–580.
7. Van Harmelen, V., S. Reynisdottir, K. Cianflone, E. Degerman, J. Hoffstedt, K. Nilsell, A. Sniderman, and P. Arner. 1999. Mechanisms involved in the regulation of free fatty acid release from isolated human fat cells by acylation stimulating protein and insulin. *J. Biol. Chem.* **274:** 18243–18251.
8. Cianflone, K., M. Maslowska, and A. D. Sniderman. 1999. Acylation stimulating protein (ASP), an adipocyte autocrine: new directions. *Semin. Cell. Dev. Biol.* **10:** 31–41.
9. Saleh, J., L. K. M. Summers, K. Cianflone, B. A. Fielding, A. D. Sniderman, and K. N. Frayn. 1998. Coordinated release of acylation stimulating protein (ASP) and triacylglycerol clearance by human adipose tissue *in vivo* in the postprandial period. *J. Lipid Res.* **39:** 884–891.
10. Summers, L. K., B. A. Fielding, V. Ilic, P. T. Quinlan, and K. N. Frayn. 1998. The effect of triacylglycerol-fatty acid positional distribution on postprandial metabolism in subcutaneous adipose tissue. *Br. J. Nutr.* **79:** 141–147.
11. Summers, L. K., B. A. Fielding, S. L. Herd, V. Ilic, M. L. Clark, P. T. Quinlan, and K. N. Frayn. 1999. Use of structured triacylglycerols containing predominantly stearic and oleic acids to probe early events in metabolic processing of dietary fat. *J. Lipid Res.* **40:** 1890–1898.
12. Bray, G. A. 1992. Pathophysiology of obesity. *Am. J. Clin. Nutr.* **55:** 488S–494S.
13. Frayn, K. N., S. W. Coppack, and S. M. Humphreys. 1993. Subcutaneous adipose tissue metabolism studied by local catheterization. *Int. J. Obes. Relat. Metab. Disord.* **17(Suppl. 3):** S18–S21.
14. Humphreys, S. M., R. M. Fisher, and K. N. Frayn. 1990. Micromethod for measurement of sub-nanomole amounts of triacylglycerol. *Ann. Clin. Biochem.* **27:** 597–598.
15. Cianflone, K., Z. Zhang, H. Vu, D. Kalant, and A. D. Sniderman. 1996. The effect of individual amino acids on apoB100 and Lp(a) secretion by HepG2 cells. *J. Biol. Chem.* **271:** 29136–29145.
16. Humphreys, S. M., and K. N. Frayn. 1988. Micro-method for preparing perchloric extracts of blood. *Clin. Chem.* **34:** 1657.
17. Maslowska, M., H. Vu, S. Phelis, A. D. Sniderman, B. M. Rhode, D. Blank, and K. Cianflone. 1999. Plasma acylation stimulating protein, adipsin and lipids in non-obese and obese populations. *Eur. J. Clin. Invest.* **29:** 679–686.
18. Bjorntorp, P. 1996. The regulation of adipose tissue distribution in humans. *Int. J. Obes.* **20:** 291–302.
19. Frayn, K. N., S. Shadid, R. Hamlani, S. M. Humphreys, M. L. Clark, B. A. Fielding, O. Boland, and S. W. Coppack. 1994. Regulation of fatty acid movement in human adipose tissue in the post-absorptive-to-postprandial transition. *Am. J. Physiol.* **266:** E308–E317.
20. Xiang, S-Q., K. Cianflone, D. Kalant, and A. D. Sniderman. 1999. Differential binding of triglyceride-rich lipoproteins to lipopotein lipase. *J. Lipid Res.* **40:** 1655–1663.
21. Shah, M., D. S. Miller, and C. A. Geissler. 1988. Lower metabolic rates of post-obese versus lean women: thermogenesis, basal metabolic rate and genetics. *Eur. J. Clin. Nutr.* **42:** 741–752.
22. Ranneries, C., J. Bulow, B. Buemann, N. J. Christensen, J. Madsen, and A. Astrup. 1998. Fat metabolism in formerly obese women. *Am. J. Physiol.* **274:** E155–E161.
23. Geissler, C. A., D. S. Miller, and M. Shah. 1987. The daily metabolic rate of the post-obese and the lean. *Am. J. Clin. Nutr.* **45:** 914–920.
24. Leibel, R. L., and J. Hirsch. 1984. Diminished energy requirements in reduced-obese patients. Metab **33:** 164–170.
25. Astrup, A. 1993. Dietary composition, substrate balances and body fat in subjects with a predisposition to obesity. *Int. J. Obes. Relat. Metab. Disord.* **17:** S32–S36.
26. Astrup, A., B. Buemann, N. J. Christensen, and S. Toubro. 1994. Failure to increase lipid oxidation in response to increasing dietary fat content in formerly obese women. *Am. J. Physiol.* **266:** E592–E599.
27. Raben, A., H. B. Andersen, N. J. Christensen, J. Madsen, J. J. Holst, and A. Astrup. 1994. Evidence for an abnormal postprandial response to a high-fat meal in women predisposed to obesity. *Am. J. Physiol.* **267:** E549–E559.
28. Hervey, G. R. 1969. Regulation of energy balance. *Nature.* **222:** 629–631.
29. Weinsier, R. L., K. M. Nelson, D. D. Hensrud, B. E. Darnell, G. R. Hunter, and Y. Schutz. 1995. Metabolic predictors of obesity. Contribution of resting energy expenditure, thermic effect of food, and fuel utilization to four-year weight gain of post-obese and never-obese women. *J. Clin. Invest.* **95:** 980–985.
30. Burstein, R., A. M. Prentice, G. Goldberg, P. R. Murgatroyd, M. Harding, and W. A. Coward. 1995. Metabolic fuel utilisation in obese women before and after weight loss. *Int. J. Obes. Relat. Metab. Disord.* **20:** 253–259.
31. de Peuter, R., R. T. Withers, M. Brinkman, F. M. Tomas, and D. G. Clark. 1992. No differences in rates of energy expenditure between post-obese women and their matched, lean controls. *Int. J. Obes. Relat. Metab. Disord.* **16:** 801–808.
32. Binnert, C., C. Pachiaudi, M. Beylot, D. Hans, J. Vandermander, P. Chantre, J. P. Riou, and M. Laville. 1998. Influence of human obesity on the metabolic fate of dietary long and medium chain triacylglycerols. *Am. J. Clin. Nutr.* **67:** 595–601.