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REVIEW

Insulin's First 100 Years - Where Next?

100th anniversary of the discovery of insulin perspective: insulin and adipose tissue fatty acid metabolism

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Abstract

Insulin inhibits systemic nonesterified fatty acid (NEFA) flux to a greater degree than glucose or any other metabolite. This remarkable effect is mainly due to insulin-mediated inhibition of intracellular triglyceride (TG) lipolysis in adipose tissues and is essential to prevent diabetic ketoacidosis, but also to limit the potential lipotoxic effects of NEFA in lean tissues that contribute to the development of diabetes complications. Insulin also regulates adipose tissue fatty acid esterification, glycerol and TG synthesis, lipogenesis, and possibly oxidation, contributing to the trapping of dietary fatty acids in the postprandial state. Excess NEFA flux at a given insulin level has been used to define in vivo adipose tissue insulin resistance. Adipose tissue insulin resistance defined in this fashion has been associated with several dysmetabolic features and complications of diabetes, but the mechanistic significance of this concept is not fully understood. This review focusses on the in vivo regulation of adipose tissue fatty acid metabolism by insulin and the mechanistic significance of the current definition of adipose tissue insulin resistance. One hundred years after the discovery of insulin and despite decades of investigations, much is still to be understood about the multifaceted in vivo actions of this hormone on adipose tissue fatty acid metabolism.

adipose tissues; adipose tissue insulin resistance; ADIPO-IR; dietary fatty acids; fatty acid metabolism; insulin; insulin resistance; nonesterified fatty acids; postprandial fatty acid metabolism

INTRODUCTION

Since its discovery 100 years ago and then its rapid introduction for the treatment of diabetes by Banting, Best, Collip, and McLeod, insulin has been primarily recognized for its role in the regulation of glucose metabolism. The glucocentric view of insulin action is entrenched in the term "diabetes mellitus," evoking the "honey urine that attracts ants" recognized since antiquity, but first officially documented in the Western world by Thomas Willis in 1674 (1). It is also inescapable by the patients and healthcare providers focusing on glycemic control to prevent the complications of diabetes. It is therefore easy to lose sight of the many other major pathophysiological metabolic actions of insulin. Suppression of plasma nonesterified fatty acid (NEFA) levels has, nevertheless, been shown in 1964 by Zierler and Rabinowitz (2) to be much more sensitive than glucose metabolism to the effect of insulin. The loss of control of NEFA metabolism by insulin plays of course a primary role in diabetic and euglycemic ketoacidosis (3–5), but has also been evoked in the past decades as an important pathogenic mechanism for insulin resistance and beta-cell dysfunction in type 2 diabetes (T2D) (6–10). Therefore, diabetes mellitus may as well have been labeled "diabetes lipidus," as suggested 18 years ago by Shafrir and Raz (11).

Adipose tissues are the major regulators of circulating NEFA and dietary fatty acids. Almost 20 years ago, our group and others have suggested abnormal regulation of adipose tissue fatty acid metabolism by insulin as a pathogenic factor in the development of T2D and its complications (7, 12). There is very wide sex, age, and ethnic-dependent variability of visceral (intra-abdominal) and subcutaneous adipose depots mass (13). These adipose depots display differences in metabolic regulation and anatomical localizations that have important implications for their association with cardiometabolic disorders (14). Increased intra-abdominal fat mass has been closely associated with the risk of T2D and cardiovascular complications (15). Genes associated with limited peripheral subcutaneous adipose tissue fat mass underpin the risk of insulin resistance, T2D and cardiovascular disease (16), supporting the expansion of peripheral adipose tissues as a mechanism to prevent excessive exposure of lean tissues to fatty acids during positive energy balance, thereby limiting the development of cardiometabolic complications (17, 18).

However attractive, the concept of impaired insulin regulation of adipose tissue fatty acid metabolism (i.e., adipose

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tissue insulin resistance) as a possible diabetogenic mechanism needs to be critically reassessed. The present review details the in vivo regulation of fatty acid metabolism in adipose tissues by insulin and their disorders in insulin-resistant states. A major emphasis is put on the regulation of postprandial plasma NEFA metabolism and adipose tissue handling of meal-derived fatty acids. Insulin and insulin resistance effects on lipoprotein metabolism at the liver and intestine are beyond the scope of the present review and have been reviewed by others elsewhere (19–21). Insulin signaling is of outstanding importance for adipose tissue development and regulation of the production of many adipokines; this topic will not be reviewed here, but is the object of excellent recent reviews (22, 23).

REGULATION OF ADIPOCYTE FATTY ACID METABOLISM BY INSULIN

The molecular mechanisms of insulin-mediated suppression of intracellular triglyceride (TG) lipolysis, the main source of adipose tissue NEFA and glycerol release, are well-described (24–26). Increased intracellular cyclic adenosine phosphate (cAMP) levels mainly from β-adrenergic stimulation activate protein kinase A that, in turn, activates adipose tissue triglyceride lipase (ATGL), hydrolyses TG and hormone-sensitive lipase (HSL), and hydrolyses diacylglycerol (DAG) to release NEFA. Insulin primarily inhibits lipolysis stimulation by activating phosphodiesterase 3B to reduce intracellular cAMP levels. Phosphatidylinositol 3-kinase (PI3K) and protein kinase B (PKB) activation by insulin also stimulates the mammalian target of rapamycin (mTORC1) that inhibits ATGL. Insulin-mediated suppression of intracellular TG lipolysis is reduced, whereas catecholamine stimulation is increased in visceral versus subcutaneous adipocytes, leading to relatively higher NEFA release from visceral adipose tissue depots (27–29). Insulin-mediated suppression of intracellular TG lipolysis is, however, not blunted ex vivo in adipocytes of obese and insulin-resistant individuals (30–32). In fact, increased basal and reduced norepinephine-stimulated adipocyte lipolysis has been recently associated with a higher future risk of developing obesity and hyperglycemia (33) and increased enzymatic deactivation of norepinephrine in human adipocytes been shown with aging (34). Pro-inflammatory cytokines, hypoxia, and mitochondrial dysfunction can also activate adipocyte lipolysis (24, 26). Therefore, the dysregulation of adipocyte intracellular TG lipolysis in insulin-resistant states is influenced by other factors than resistance to the action of insulin per se.

Insulin also promotes adipocyte fatty acid storage into TG by different mechanisms. First, insulin stimulates fatty acid re-esterification (35) by increasing glucose uptake that supplies glyceroneogenesis to provide glycerol for the synthesis of TG (36), and through the stimulation of diacylglycerol acyltransferase that catalyzes TG esterification (37, 38). Second, insulin stimulates adipocyte de novo lipogenesis directly through mTOR-mediated stimulation of sterol regulatory element-binding protein (SREBP1c) and indirectly through glucose-mediated stimulation of carbohydrate-responsive element-binding protein (ChREBP) (24). In effect, adipocyte fatty acid esterification and lipogenesis stimulation by insulin is critically dependent on glucose availability (39–41). Insulin regulates adipogenesis over the long-term at the transcriptional level through modulation of SREBP1c, USF-1, and LXR-α (42). Adipocytes from insulin-resistant individuals display reduced lipogenic gene expression and a lower fatty acid esterification rate in response to insulin (24, 43). Interestingly, ATP production is necessary to sustain lipogenesis and, therefore, adipocyte mitochondrial dysfunction has been evoked to explain reduced lipogenesis in insulin-resistant states (24). Adipocyte respiration is, however, not necessarily reduced in insulin-resistant states (44, 45) and insulin signaling does not acutely regulate adipocyte respiration (39, 46). Impaired adipocyte glucose metabolism is therefore the most likely mechanism leading to reduced adipocyte lipogenesis in insulin-resistant states.

INSULIN EFFECTS ON SYSTEMIC NEFA FLUXES IN VIVO

In Vivo Inhibition of Intracellular TG Lipolysis and Stimulation of Fatty Acid Esterification by Insulin in Adipose Tissues

The predominant in vivo effect of insulin on NEFA metabolism is the suppression of the NEFA appearance rate (Ra_{NEFA}) from inhibition of adipose tissue intracellular TG lipolysis. Intravenous NEFA tracer methodologies employed with single or multistep euglycemic hyperinsulinemic clamp are considered the gold-standard to measure the effect of insulin on this process (47). Another proposed gold-standard to measure insulin-mediated suppression of adipose tissue lipolysis is the suppression of glycerol appearance rate ($Ra_{glycerol}$). This method rests on the following three premises: 1) as opposed to NEFA, there is no reuptake of glycerol by adipose tissues following intracellular lipolysis, due to the low adipose tissue activity of glycerol kinase (48); 2) the fraction of circulating glycerol accounted for by the conversion of glucose to glycerol (i.e., glyceroneogenesis) is ∼1% in the fasting state and ∼7% even during glucose infusion, without change in absolute glyceroneogenesis-mediated rates of appearance of glycerol from the fasting state to the intravenous administration period (49); and 3) the metabolic clearance rate of plasma glycerol is unaffected by insulin (50).

Insulin administration typically results in more than 60% suppression of Ra_{NEFA} but only 40–50% suppression of $Ra_{glycerol}$ in the fasting state (51). From the results obtained in adipocytes, insulin-mediated suppression of Ra_{NEFA} may result from the suppression of intracellular TG lipolysis plus the stimulation of fatty acid re-esterification, whereas $Ra_{glycerol}$ would be a measure of the former process only. Therefore, the gap between insulin-mediated suppression of Ra_{NEFA} and $Ra_{glycerol}$ has been previously assumed to be caused by insulin-mediated stimulation of the adipose tissue NEFA re-esterification rate (35, 52–54). This interpretation has been supported by arteriovenous gradient measurements with isotopic tracers during a euglycemic hyperinsulinemic clamp in the fasting state showing that subcutaneous adipose tissue shuts down its intracellular lipolysis and export of NEFA, increases its import of glucose, and re-esterifies most of the NEFA produced from the residual intracellular lipolysis (55). However, adipose tissue NEFA

uptake is not stimulated by insulin in the fasting state using the NEFA tracer bolus followed by the adipose tissue biopsy method (56). $Ra_{glycerol}$ can also be produced from intravascular lipolysis of TG-rich lipoproteins and intracellular TG lipolysis in muscles. Thus, $Ra_{glycerol}$ is an index of total lipolysis and is less reliable for the quantification of adipose tissue intracellular TG lipolysis, especially in the postprandial state. The use of simultaneous intravenous NEFA and glycerol tracers is therefore unreliable to measure the in vivo adipose tissue NEFA re-esterification rate.

Insulin Regulation of Postprandial NEFA Fluxes and NEFA Spillover

The regulation of postprandial Ra_{NEFA} is complex (Fig. 1) as it stems from the sum of NEFA produced by intracellular adipose tissue lipolysis and NEFA produced from the intravascular hydrolysis of chylomicron TG (57–59) and, to a lesser extent, from VLDL-TG (60), that are not trapped, or are trapped and released very quickly by the TG-fatty acid re-esterification/lipolysis cycle in tissues (57–59). The later process, NEFA spillover, originates to a large extent from adipose tissues, with a minor contribution from skeletal muscles (57, 59) and none from the heart and liver (61–63).

NEFA spillover has been assessed in humans using a variety of methods including (Table 1): 1) single meal fatty acid tracer; 2) dual meal + intravenous fatty acid tracers; 3) intravenous TG + NEFA tracers; and 4) arteriovenous gradient across abdominal subcutaneous adipose tissue and/or splanchnic circulation and/or other anatomic regions or organs. With one exception (78), no study has directly compared these methods, but there appears to be no major difference between them in the range in fractional NEFA spillover reported in healthy individuals. However, the range of fractional NEFA spillover reported varies considerably between studies (i.e., 10–77%) (59, 64, 65, 68, 69, 74, 75, 78, 80). One study demonstrated that the spillover rates of palmitate, oleate, and linoleate are similar (74). NEFA spillover tends to be larger with larger meal fat content (71), emulsified meal fat (75), and after the first meal of the day (68). The fractional NEFA spillover rate also varies in different adipose tissue depots, being higher in the splanchnic (~60%) versus systemic circulation (~24%) (61).

It is possible that insulin stimulation of adipose tissue fatty acid esterification rate may decrease postprandial Ra_{NEFA} by reducing NEFA spillover in addition to its effect on intracellular TG lipolysis. NEFA spillover from dietary fatty acids peaks between 300 and 360 min after meal intake (64), at a time when plasma insulin concentration often reverts to the fasting level (Fig. 1). Both the systemic and splanchnic fractional NEFA spillover rates are unchanged from fasting to the postprandial state using intravenous TG + NEFA tracers (61), suggesting proportional reduction of Ra_{NEFA} from intracellular TG lipolysis and NEFA spillover by postprandial insulin. In overweight or obese subjects under continuous feeding with saline versus hyperinsulinemic clamp at matched hyperglycemia levels, Muthusamy et al. (70) found virtually no change in the fractional NEFA spillover rate. However, the two conditions displayed 2.6- versus 6.3-fold elevation in plasma insulin levels from the fasting state. From that study, it was therefore not possible to rule out that insulin may have an additional effect on NEFA spillover from fasting to low postprandial insulin level range. Using intravenous saline versus nicotinic acid, an inhibitor of intracellular TG lipolysis, during continuous feeding, Nelson et al. (69) demonstrated a significant reduction in fractional NEFA spillover, suggesting that NEFA spillover stems at least in part from the intracellular TG lipolysis/NEFA re-esterification cycle. We used nicotinic acid to independently suppress adipose tissue intracellular lipolysis during a euglycemic hyperinsulinemic clamp with an intravenous

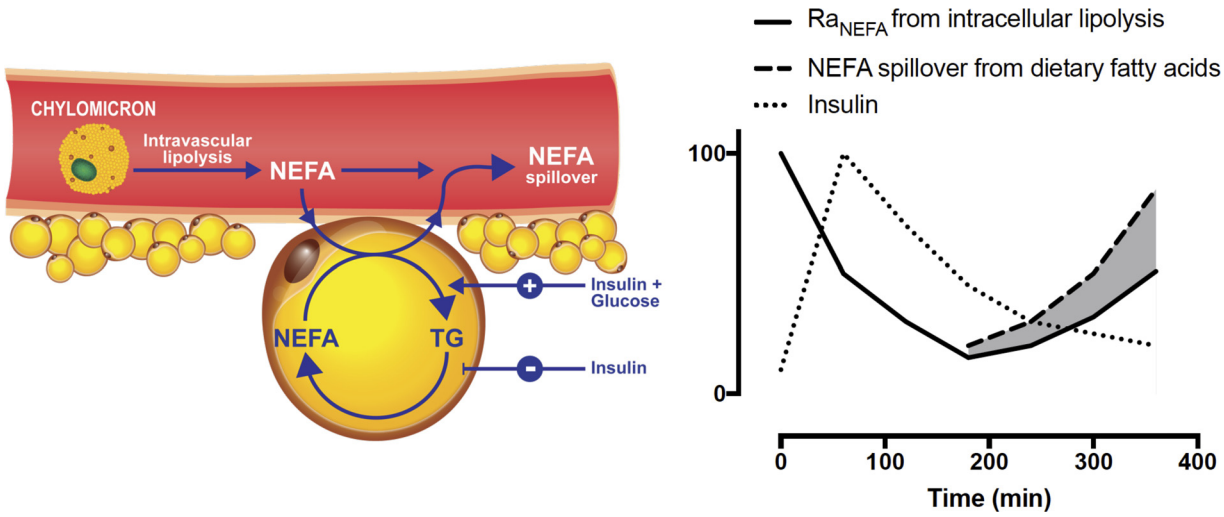

Figure 1. Mechanism of meal-derived nonesterified fatty acid (NEFA) spillover and its regulation by insulin (*left*). Insulin limits NEFA spillover by suppressing intracellular triglyceride (TG) lipolysis, which interrupts the esterification/lipolysis cycling of newly stored meal-derived NEFA, preventing their rapid release into the circulation after the meal. Insulin-mediated stimulation of adipose tissue NEFA esterification occurs and is detectable in vivo in the presence of hyperglycemia. Relative (expressed as percentage) systemic NEFA appearance rate (Ra_{NEFA}) from intracellular TG lipolysis and NEFA spillover after a meal (*right*). Ra_{NEFA} from intracellular TG lipolysis is rapidly and profoundly suppressed by postprandial insulin elevation and meal-derived NEFA spillover gradually increases between 2 and 6 h after the meal, concomitantly with the decrease in insulin level and the release of its inhibition of intracellular TG lipolysis.

Table 1. *Assessment of NEFA spillover from published studies in humans*

Reference	Population	Intervention	Method	Insulin Levels	% NEFA Spillover		Comment
					Cont.	Int.	
Roust 1993 (64)	Non-obese women *vs.* lower body obese women *vs.* upper body obese women		Fasting + postprandial iv [2-^{3}H]oleate with oral [^{14}C]triolein	Lowest *vs.* Intermediate *vs.* Highest postprandial level	~30 to 40 in the 3 groups		Estimated from graphical data; no difference between groups
Miles 2004 (59)	Healthy lean men		Fasting state iv [^{3}H]triolein, [^{14}C]glycerol and [^{14}C]oleate	Fasting level	36		
Barrows 2005 (65) Timlin 2005 (66)	Healthy lean men	Meal *vs.* continuous enteric feeding	Enteric [31-^{2}H]glyceryl trihexadecanoate and iv [^{13}C]hexadecanoate	Higher *vs.* lower postprandial level	24	11*	
Tan 2005 (67)	Men and women with T2D	Randomized, crossover intervention, placebo *vs.* rosiglitazone 4 mg bid for 12 weeks	Fasting and postprandial iv [1,1,1-^{13}C]tripalmitine in meal with AV difference across forearm and abdominal SCAT	Lower fasting and postprandial levels with rosiglitazone	ND	ND	Rosiglitazone increased fasting AT NEFA release and AT blood flow, reduced NEFA spillover, but did not change postprandial SCAT fatty acid uptake and release
Nelson 2007 (61)	Obese men and women	Fasting *vs.* postprandial	AV difference from artery, systemic vein and hepatic vein with iv [^{3}H]triolein, [^{13}C]- or [^{14}C]oleate	Fasting *vs.* postprandial levels	38	34	Splanchnic (54 to 60% range) significantly greater than systemic NEFA spillover
Nelson 2007 (63)	Non-diabetic subjects with coronary artery disease		Fasting state AV difference across arterial and coronary sinus circulation with iv [^{14}C]oleate and [^{3}H]triolein	Fasting level	49		Cardiac NEFA spillover was 35%
Ruge 2009 (68)	Healthy lean men		iv [^{2}H]palmitate over 24 h with 3 meals containing [^{13}C]linoleate (breakfast), [^{13}C]oleate (lunch) and [^{13}C]palmitate (dinner) with AV difference across SCAT	No difference in insulin AUC between meals	77	34*	NEFA spillover greater at breakfast *vs.* dinner
Nelson 2012 (69)	Healthy lean men and women	Intravenous saline *vs.* nicotinic acid	Continuous feeding with iv [U-^{13}C]oleate and [^{3}H]trioleate infusions	Fourfold increase in postprandial insulin levels in overweight or obese participants; no change with nicotinic acid	29	21*	
	Overweight or obese men and women				29	21*	
Muthusamy 2012 (70)	Overweight or obese men and women	Intravenous saline *vs.* matched glycemic-hyperinsulinemic clamp	Continuous feeding with iv [U-^{13}C]oleate and [^{3}H]trioleate infusions	2.6- *vs.* 6.3-fold increase in insulin level from fasting with saline *vs.* insulin clamp	25	26	

Continued

Table 1.— *Continued*

Reference	Population	Intervention	Method	Insulin Levels	% NEFA Spillover		Comment
					Cont.	Int.	
Puga 2012 (71)	Healthy older non-obese men	0.4 *vs.* 0.7 g/kg meal fat. Almost only fat in both meals	4 *vs.* 7 mg [^{13}C]triolein per kg body weight orally	No difference in post-prandial insulin levels	ND	ND	More than 2-fold increase in NEFA spillover with higher meal fat content; NEFA spillover inversely related to adiposity with higher fat meal
Alligier 2013 (72)	Healthy non-obese men	56-day overfeeding	*d*31-palmitate meal test		ND	ND	30% increase in NEFA spill-over from meal with over-feeding; correlated with increase in visceral fat mass
Almandoz 2013 (73)	Men and women with T2D	Lifestyle change with 15 kg weight loss on average	Continuous feeding with iv [U-^{13}C]oleate and [^{3}H]trioleate infusions	Significant reduction in postprandial insulin levels after weight loss	28	31	NEFA spillover inversely associated with both leg and trunk fat masses, but positively associated with trunk-to-leg fat mass ratio
Nelson 2013 (74)	Healthy lean men and women	Meal with normal *vs.* high palmitate content	Oral [^{3}H]trilinolein, [^{13}C] triolein and [^{13}C]tripal-mitin + iv infusion of [^{14}C]palmitate, [^{13}C] oleate and [^{13}C] linoleate	Not reported	23 *vs.* 18 *vs.* 15	16 *vs.* 13 *vs.* 25†	Palmitate *vs.* oleate *vs.* lino-leate; linoleate spillover higher with high palmitate meal
Vors 2013 (75)	Healthy lean men Obese men	Spread *vs.* emulsi-fied meal fat	Oral [^{13}C]tripalmitin, [^{13}C] triolein and [^{13}C]trioc-tanoin (medium chain fatty acid)	No significant change in postprandial insulin level between meals	1110	19* 15*	
Noll 2015 (76)	Men and women with impaired glucose tolerance	7-day caloric + sat-urated fat restriction	Fasting + postprandial iv *d*4-palmitate with oral [U-^{13}C]palmitate	Significant reduction in postprandial insulin levels	30	30	
Grenier-Larouche 2017 (77)	Men and women with morbid obesity and T2D	Before *vs.* 3 days *vs.* 3 months *vs.* 12-month after bil-iopancreatic diversion with du-odenal switch	Fasting iv intralipid + heparin and [^{3}H]trio-lein with [^{13}C]palmi-tate and [^{13}C]glycerol during an euglyce-mic-hyperinsulinemic clamp	Reduction in fasting plasma insulin levels 3-days, and 3 and 12-months after surgery	41	25* 39 37	Increase in NEFA spillover 12-months after surgery associated with reduced SCAT cell size
Grenier-Larouche 2017 (77)	Men and women with morbid obesity, but without T2D	Before *vs.* 3 days *vs.* 3 months *vs.* 12-month after bil-iopancreatic diversion with du-odenal switch	Fasting iv Intralipid + heparin and [^{3}H]trio-lein with [^{13}C]palmi-tate and [^{13}C]glycerol during an euglyce-mic-hyperinsulinemic clamp	Reduction in fasting plasma insulin levels 3-days, and 3 and 12 months after surgery	37	17* 23 49	Increase in NEFA spillover 12-months after surgery associated with reduced SCAT cell size
Piché 2018 (78)	Healthy men and women with BMI $\leq$ 25*vs.* BMI $>$ 25 kg/m^2		Oral U-^{13}C-palmitate	Non-significantly higher postprandial insulin levels with high BMI	22 vs. 19†		NEFA spillover inversely associated with BMI, waist girth, HOMA-IR and fasting TG; no difference in postprandial TG levels

Continued

Table 1.— *Continued*

Reference	Population	Intervention	Method	Insulin Levels	% NEFA Spillover		Comment
					Cont.	Int.	
Piché 2018 (78)	Age and BMI-matched men *vs.* women		Oral [U-^{13}C]palmitate	Non-significantly higher postprandial insulin levels in men	20 vs. 30†		Higher postprandial TG and waist girth in men
Piché 2018 (78)	Age and BMI-matched men *vs.* women		Oral [U-^{13}C]palmitate + AV difference across SCAT	Non-significantly higher postprandial insulin levels in men	10 vs. 29†		
Carreau 2020 (79)	Men and women with morbid obesity and T2D	Before and 8 to 12 days after bariatric surgery	Fasting + postprandial iv *d4*-palmitate with oral [^{13}C]palmitate	Significant reduction in insulin levels	28	15*	Reduction in NEFA spillover associated with reduction in HOMA-IR
Noll 2020 (80)	Healthy lean or overweight men and women	7-day overfeeding	Fasting + postprandial iv *d4*-palmitate with oral [^{13}C]palmitate	Significant increase in postprandial insulin levels	28	35	Significant increase in SCAT dietary fatty acid storage with reduction in cardiac and skeletal muscle partitioning

AT, adipose tissue; AUC, area under the curve; AV, arteriovenous; BMI, body mass index; Cont., control condition; HOMA-IR, homeostatic model assessment of insulin resistance; Int., intervention; iv, intravenous; ND, not determined; NEFA, nonesterified fatty acids; SCAT, subcutaneous adipose tissue; T2D, type 2 diabetes; TG, triglycerides.
*Significantly different than control condition; †Significantly different between groups.

lipid emulsion + heparin administration to determine whether there is any residual effect of fasting versus clamp insulin levels on Ra_{NEFA} through a putative stimulation of NEFA esterification rate (81); we found that suppression of intracellular lipolysis accounts for at least 60–70% of insulin-mediated suppression of palmitate appearance and oxidation rates during enhanced intravascular TG lipolysis. Likewise, insulin infusion after a meal does not further increase the postprandial inhibition of intracellular lipolysis or stimulation of lipoprotein lipase (LPL) activity, but increases the apparent esterification rate of meal fatty acids in adipose tissues assessed by the abdominal adipose tissue arteriovenous gradient technique (82). In healthy subjects, Ra_{NEFA} during continuous feeding with or without an insulin clamp are marginally superior to Ra_{NEFA} during an insulin clamp in the fasting state (51).

These evidences demonstrate that insulin-mediated stimulation of fatty acid esterification in adipose tissues plays a minor role in limiting postprandial Ra_{NEFA} in vivo. Insulin-mediated suppression of intracellular lipolysis is the main mechanism that limits Ra_{NEFA} from both the pre-stored adipose tissue TG and from the spillover of meal NEFA that are esterified but then rapidly released back through disinhibition of lipolysis by reduced insulin levels 3 to 6 h after the meal (Fig. 1).

Insulin Regulation of Adipose Tissue Fatty Acid Storage

In the postprandial state, there is a clear increase of adipose tissue uptake of meal-derived fatty acids from circulating TG-rich lipoproteins (57, 68, 83, 84) that are preferentially extracted compared to plasma NEFA (57). The uptake of meal-derived fatty acids per volume of adipose tissue is higher in intra-abdominal, intermediate in abdominal subcutaneous and lower in gluteal and femoral subcutaneous adipose depots (85–88) and peaks 180 to 240 min after meal intake (86). Insulin can activate adipose tissue lipoprotein lipase activity in addition to intracellular esterification pathways, contributing to enhance adipose tissue storage of meal fatty acids (89). However, adipose tissue extraction of fatty acids either from circulating TG or from the NEFA pools does not increase during a euglycemic hyperinsulinemic clamp in the fasting state (56, 90). There is in vivo evidence from studies in rodents that the fatty acid esterification rate at the liver is driven by fatty acid availability, but is not stimulated by insulin signaling (91). Indeed, adipose tissue uptake of fatty acids from circulating TG follows closely the appearance rate of TG-rich lipoproteins, not postprandial insulin levels (92). Furthermore, net adipose tissue uptake of fatty acids from circulating TG and reduced intracellular lipolysis can occur without increase in circulating insulin during intravenous or oral fat administration (93, 94). In these conditions, however, apparent adipose tissue esterification of meal fatty acids from arteriovenous gradient measurements is not stimulated (93). Insulin also does not directly contribute to the postprandial stimulation of adipose tissue blood flow that could contribute to enhance lipid storage (95). Thus, factors other than insulin play more important roles to stimulate adipose tissue uptake and storage of meal fatty acids including meal fat content (96), rate of meal fat appearance in circulation (65), repeated meal intake (68), lower body fat distribution (96,

97), sex hormones (98), and other postprandial hormonal responses such as that of glucose-dependent insulinotropic polypeptide (GIP) (99).

Insulin Effect on Plasma NEFA Clearance Rate

It is also possible that insulin directly affects the tissue clearance of plasma NEFA. Indeed, in vitro evidence suggests that insulin can stimulate the cell membrane translocation of fatty acid transporters such as CD36 (100, 101). During euglycemic hyperinsulinemic clamp or in the postprandial state, there is a consistent elevation of plasma NEFA metabolic clearance rate (i.e., Ra_{NEFA}/plasma NEFA concentration) (81, 102, 103). Using a multi-compartmental modeling approach, postprandial reduction of plasma NEFA is best explained by both insulin-mediated reduction in Ra_{NEFA} and insulin-stimulated uptake of NEFA (104). In rats, insulin, however, leads to increased clearance of plasma NEFA in adipose tissues only in some (105) but not all studies (106). In pigs, increased uptake of circulating NEFA into adipose tissues is only detected after hyperglycemic hyperinsulinemic, not euglycemic hyperinsulinemic clamp, which contrasts with stimulation of adipose tissue uptake of glucose in both conditions (107). This is consistent with the known stimulation of adipocyte TG synthesis and NEFA re-esterification with enhanced glucose availability (40, 41) or with differential local adipose tissue blood flow regulation during hyperglycemia (53).

We tested experimentally whether insulin per se affects systemic NEFA clearance rate over a wide physiological range of plasma NEFA concentrations using intravenous lipid + heparin without and with nicotinic acid and normo versus hyperinsulinemic clamp (50). We found that NEFA clearance was linearly and inversely related to Ra_{NEFA} without any effect of insulin level per se on NEFA clearance if Ra_{NEFA} was experimentally maintained at the same rate by exogenous lipid infusion. An inverse log-linear relationship between NEFA levels and NEFA clearance was also reported by others (74). Thus, the calculated NEFA clearance (i.e., Ra_{NEFA}/[NEFA]) increases linearly with the reduction of Ra_{NEFA} (50), creating an artifactual increase in NEFA clearance during hyperinsulinemia because of the insulin-mediated reduction in Ra_{NEFA}.

Summary of Insulin-Mediated Regulation of Adipose Tissue NEFA Fluxes in Vivo

The mechanism of suppression of postprandial Ra_{NEFA} by insulin in the healthy state is mostly through suppression of intracellular TG lipolysis in adipose tissues. Postprandial suppression of NEFA spillover by insulin occurs essentially via the interruption of intracellular TG-NEFA re-esterification/lipolysis cycling through the suppression of adipose tissue intracellular TG lipolysis (Fig. 1). Although an insulin stimulatory effect on NEFA esterification in adipose tissues exists, this mechanism plays a minor role, if any, in vivo under physiological conditions to limit postprandial NEFA spillover. The apparent insulin-mediated stimulation of systemic NEFA clearance during insulin clamp is artifactual and caused by the non-linear relationship between NEFA levels and Ra_{NEFA}. Postprandial adipose tissue extraction of meal fatty acids is mostly regulated by factors other than insulin under physiological conditions. There is, however, evidence for an acute stimulation of NEFA storage in adipose tissues during combined hyperglycemia + hyperinsulinemia, compatible with the dependence of insulin-mediated stimulation of adipose tissue glyceroneogenesis and TG synthesis on glucose availability.

ADIPOSE TISSUE FATTY ACID METABOLISM IN INSULIN-RESISTANT STATES

Assessment of Adipose Tissue Insulin Sensitivity

In vivo methods to determine adipose tissue insulin sensitivity are mostly based on insulin-mediated suppression of adipose tissue lipolysis. Inhibition of Ra_{NEFA} during a multistep euglycemic hyperinsulinemic clamp may be considered the "gold-standard" to measure adipose tissue insulin sensitivity in vivo, but ADIPO-IR (i.e., fasting plasma NEFA x circulating insulin levels) is much more frequently employed and displays good correlation with this more robust, but less conveniently applicable method (47). Low-dose insulin clamp-mediated suppression of $Ra_{glycerol}$, another proposed "gold-standard" measure, is also very well-correlated with ADIPO-IR or other variants of adipose tissue insulin sensitivity using fasting or insulin clamp-derived indices, all displaying high areas under the receiver-operator characteristic curve to identify subjects with severe adipose tissue insulin resistance using the glycerol tracer method (108).

Recently, a combined PET-magnetic resonance imaging (MRI) whole-body acquisition with [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) administration during a euglycemic hyperinsulinemic clamp was developed to determine tissue-specific insulin sensitivity of glucose uptake (109). Glucose uptake of intra-abdominal and subcutaneous adipose tissues correlated well with whole-body insulin sensitivity and was reduced in subjects with prediabetes or T2D (110). This new method offers an alternative, more glucocentric method to assess adipose tissue insulin sensitivity, but the relationship between NEFA versus glucose-based methods to assess adipose tissue insulin sensitivity has not been determined thus far.

Although this is beyond the scope of the present review, it should be acknowledged that the concept of adipose tissue insulin resistance may also extend to insulin resistance of adipose tissue immune, vascular, and connective tissue cells that in turn could influence adipose tissue biology and systemic metabolism (22, 111–113). Elucidating the role of insulin resistance of stoma vascular cells in different adipose tissue depots on the regulation of fatty acid metabolism in vivo in humans needs further studies.

Thus, adipose tissue insulin sensitivity could be defined in multiple ways. Herein, adipose tissue insulin sensitivity and resistance will refer to the insulin-mediated suppression on plasma NEFA levels and/or systemic flux (Fig. 2).

Adipose Tissue Insulin Sensitivity in T2D and Insulin-resistant States

There is a very large body of experimental evidence showing that increased NEFA flux leads to the development of insulin resistance and beta-cell dysfunction within hours (6). Tissue lipotoxicity may also be involved in many T2D

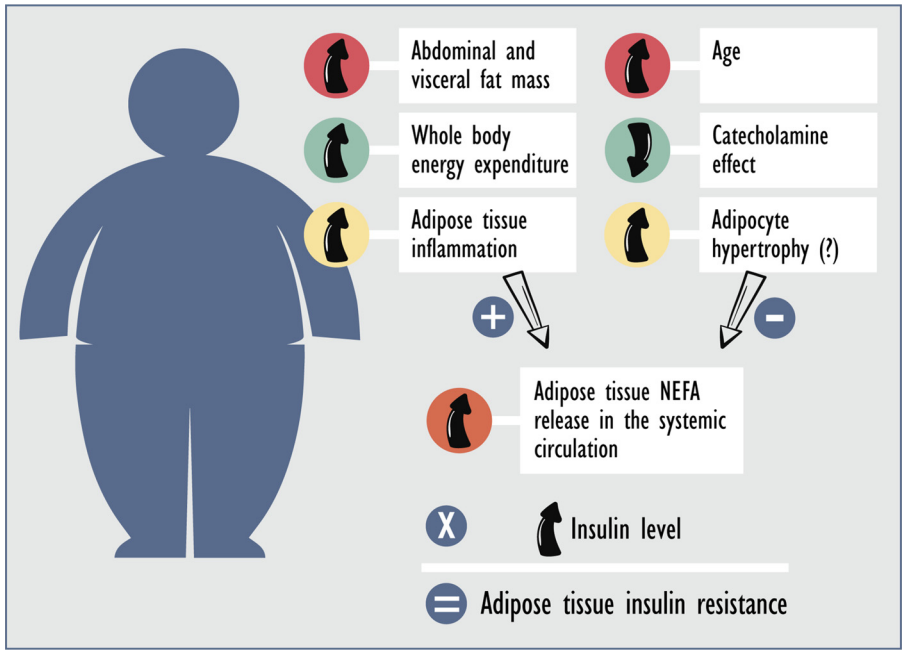

Figure 2. The concept of adipose tissue insulin resistance based on abnormal systemic nonesterified fatty acid (NEFA) metabolism. The most prevalent definition of adipose tissue insulin resistance uses plasma NEFA level (or flux) times plasma insulin levels. However, factors such as increased abdominal fat mass and age-mediated reduction in catecholamine-stimulated intracellular triglyceride lipolysis confound this measurement.

complications (114–117). Thus, a role for adipose tissue insulin resistance in the progression from health to T2D and complications could be expected. ADIPO-IR indeed progressively increases from healthy to impaired glucose tolerance to T2D, in association with declining beta-cell function in adults (118) and adolescents (119). Insulin-mediated inhibition of lipolysis and insulin-stimulated lipogenesis in subcutaneous abdominal adipose tissue biopsy samples are also inversely related to cardiovascular risk (120). Similar to glucose-dependent measures of beta-cell function, best described as a hyperbolic relationship between insulin secretion and sensitivity (i.e., the insulin disposition index) (121), fasting ADIPO-IR also displays a hyperbolic relationship with glucose-stimulated insulin secretion (GSIS) (122). In the later study, ADIPO-IR x GSIS, an "adipose tissue disposition index," was strongly correlated with plasma glucose at fasting and at 2 h after an oral glucose tolerance test. ADIPO-IR is also inversely related with insulin clearance (123), which is impaired in insulin-resistant states and T2D.

Visceral fat and ectopic TG deposition in lean organs, especially in the liver, are closely associated with cardiometabolic risk (14, 15, 124–127). Adipose tissue-derived NEFA flux accounts for the majority of fatty acids to the hepatic intracellular and VLDL-TG pools (66, 128–130). Therefore, it is expected that adipose tissue insulin resistance would be an important determinant of liver TG content and fatty acid metabolism in insulin-resistant states and T2D. Fasting ADIPO-IR is indeed independently associated with liver fat content (131) and visceral fat (119). There is a linear relationship between insulin-mediated suppression of plasma NEFA during a low-dose euglycemic hyperinsulinemic clamp and liver fat content in non-diabetic and diabetic middle-aged overweight and obese individuals (132). The splanchnic flux of NEFA from the "portal (i.e., mesenteric and omental) adipose tissues" (133) is of particular interest because it is directly available to the liver and may more directly contribute to hepatic steatosis, insulin resistance, and increased hepatic glucose production (134). This "portal theory" of hepatic insulin resistance has been investigated by many groups over the past decades (133, 135, 136). Insulin-mediated suppression of splanchnic Ra_{NEFA} is less than that of the legs in healthy subjects (137). Splanchnic Ra_{NEFA} is higher in viscerally obese subjects with T2D during a hyperglycemic hyperinsulinemic clamp (138) and is selectively increased in insulin-resistant, abdominally obese individuals, being an important source for the greater VLDL-TG secretion in these individuals (139). However, there is also evidence for impaired VLDL-TG secretion, and/or a combination of increased de novo lipogenesis and impaired hepatic fatty acid oxidation as mechanisms for liver TG accumulation in dysmetabolic states (140–144). Therefore, multiple mechanisms may contribute to liver steatosis in addition to adipose tissue insulin resistance.

Adipose tissue insulin sensitivity is, however, not invariably altered in dysmetabolic states. For example, insulin clamp-mediated reduction of plasma NEFA levels was similar in patients with impaired fasting glucose versus healthy controls displaying the same excess of body fat mass in one controlled study (145); in the latter study, only hepatic, not skeletal muscle or adipose tissue insulin sensitivity was reduced with impaired fasting glucose. Likewise, fasting $Ra_{palmitate}$, its oxidation rate, and their suppression during an insulin clamp was normal in first-degree relatives of patients with T2D (51). Equally obese patients with versus without T2D had exactly the same fasting and insulin clamp-mediated suppression of Ra_{NEFA} and $Ra_{glycerol}$ before and after bariatric surgery in another study (77). In contrast, subjects with T2D having higher visceral fat mass, but with otherwise matched body fat and lean mass were found to have higher systemic, splanchnic, and leg release of palmitate during a hyperglycemic hyperinsulinemic clamp versus healthy subjects (138). Fasting and postprandial $Ra_{palmitate}$

and oxidation rate were shown to be elevated without or with concomitant euglycemic hyperinsulinemic clamp in patients with overt T2D (51). In the latter study, the greater NEFA flux in T2D was associated with excess abdominal fat mass, hyperglycemia, and resting energy expenditure. Resting energy expenditure was found to be positively associated with systemic NEFA flux, a relationship that was shifted upward in women (146). Weight gain with overfeeding has been associated with deterioration of both insulin sensitivity of glucose metabolism and adipose tissue insulin sensitivity in many studies (147–150). When sex, age, and BMI are matched, patients with T2D displayed normal post-oral glucose tolerance test (OGTT) plasma glycerol response and similar insulin-mediated inhibition of ex vivo adipose tissue lipolysis, but increased plasma NEFA response independently associated with chronic hyperglycemia, visceral adipose tissue mass, and male sex (151). Ra_{NEFA} per volume of adipose tissue was shown to be reduced in insulin-resistant individuals because of their increased plasma insulin level (152, 153) and lower response to catecholamines (154). Aging, which is usually associated with increased visceral and total adipose tissue mass (13), reduces catecholamine-stimulated adipose tissue lipolysis, likely due to local enzymatic degradation of norepinephrine (34, 155).

In summary, excess fat mass, especially abdominal obesity, in addition to age, sex, and energy expenditure are important confounders of circulating NEFA flux (64, 146) and should be taken into account to interpret the increase in ADIPO-IR and other markers of adipose tissue insulin resistance based on NEFA metabolism in patients with insulin resistance and T2D. "Adipose tissue insulin resistance" based on excess NEFA mobilization from adipose tissues reflects the combined influence of increased visceral and abdominal adipose tissue mass, whole-body energy requirement, age-related reduction in norepinephrine signaling and several other potential sex- and metabolic status-related changes in other hormones and cytokine signaling in addition to any adipocyte resistance to insulin anti-lipolytic action (Fig. 2).

Insulin-Mediated Regulation of Plasma NEFA Spillover in Obesity and Insulin Resistance

Postprandial Ra_{NEFA} and $Ra_{glycerol}$ are elevated in obese, insulin-resistant individuals with or without T2D (51, 64, 102, 156). A specific defect in the postprandial regulation of NEFA spillover has been proposed as a source for postprandial elevation of plasma NEFA in insulin-resistant states (7). Using combined administration of nicotinic acid, insulin clamp, and intravenous lipids, we found that insulin-mediated suppression of the palmitate appearance rate not accounted by suppression of intracellular lipolysis was fully abolished in first-degree relatives of patients with T2D (157), suggesting blunted insulin-mediated suppression of NEFA spillover from adipose tissues. However, the studies that directly compared obese versus lean individuals did not find a difference in fractional NEFA spillover from meal or intravenous TG tracers (64, 69, 75) or even found a reduction of NEFA spillover in obese individuals (78) (Table 1). Three studies indeed reported an inverse association between total body and/or leg or trunk fat mass and fractional NEFA spillover (71, 73, 78). There is also no apparent increase in fractional NEFA spillover in individuals with insulin resistance or T2D (Table 1). Roust and Jensen (64) found no difference in NEFA spillover in insulin-resistant upper body versus lower body obese or lean women. Likewise, Nelson et al. (69) reported virtually identical fractional NEFA spillover in otherwise healthy obese versus lean individuals despite a fourfold increase in postprandial insulin levels in the former. Using intravenous TG and NEFA tracers during intralipid + heparin administration during a euglycemic hyperinsulinemic clamp, we also found similar NEFA spillover in morbidly obese patients without or with T2D before bariatric surgery, despite increased insulin resistance in the latter group (77).

Results from studies testing interventions that reduce insulin resistance are mixed with regards to their effect on NEFA spillover (Table 1). Four-week treatment with rosiglitazone resulted in improvement in insulin sensitivity, reduction in postprandial insulin levels, and reduction in NEFA spillover versus placebo in patients with T2D (67). Almandoz et al. (73), however, reported no change in fractional NEFA spillover during continuous feeding after a lifestyle intervention resulting in 15 kg weight loss on average and significant reduction in postprandial insulin levels in patients with T2D. Similarly, we found no change in fractional NEFA spillover in a randomized crossover study comparing a 7-day low-calorie and low-saturated fat diet versus control diet that led to significant reduction in postprandial insulin levels in subjects with impaired glucose tolerance (76). Using intravenous TG + NEFA tracers with intralipid + heparin administration during a euglycemic hyperinsulinemic clamp, we found significant reduction in fractional NEFA spillover together with reduced plasma insulin levels and HOMA-IR 3 days after bariatric surgery in morbidly obese individuals with or without T2D (77). Interestingly, despite important weight loss and continuing metabolic improvement, fractional NEFA spillover gradually increased back to pre-surgical levels at 3 and then 12 months after surgery in these individuals, closely following the reduction in abdominal subcutaneous adipocyte size (77). We recently confirmed the significant reduction in fractional NEFA spillover early (8 to 12 days) after bariatric surgery in patients with T2D, this time using dual meal + intravenous labeling techniques (79). This reduction in NEFA spillover was directly associated with the reduction of HOMA-IR after surgery.

Limited data exist on change in NEFA spillover from overfeeding studies that lead to weight gain and deterioration of insulin sensitivity (Table 1). We found a nonsignificant increase in fractional NEFA spillover in a randomized crossover 7-day overfeeding versus isocaloric diet intervention that led to a significant increase in postprandial insulin levels in healthy subjects (80). Overfeeding for 56 days in healthy individuals led to marginal elevation of homeostatic model assessment of insulin resistance (HOMA-IR) and increased postprandial NEFA spillover associated with increased visceral fat mass (72).

In summary, cross-sectional comparisons of subjects with varying degrees of insulin resistance do not support an insulin resistance-mediated defect of the regulation of NEFA spillover. Intervention studies that lead to change in insulin sensitivity, however, offer a mixed picture. One important confounder appears to be the variation of visceral adipose tissue mass. Because change in visceral adipose tissue mass

can be dissociated from change in total body mass and can be differentially affected by various interventions (158), this may have confounded the interpretation of the studies discussed above. Change in subcutaneous adipocyte size is another potential factor affecting NEFA spillover that needs to be further studied (77).

Insulin-Mediated Regulation of Adipose Tissue Fatty Acid Storage in Insulin-Resistant States

In many studies, obese, hyperinsulinemic individuals displayed blunted inhibition of intracellular lipolysis and reduced stimulation of intravascular TG lipolysis and meal fatty acid storage *per* volume of subcutaneous adipose tissues after meal intake (159–161), in contrast to normal meal-induced adipose tissue glucose uptake (162). Postprandial stimulation of adipose tissue blood flow, which could perhaps contribute to blunt meal fat storage, is also impaired in an insulin-resistant state (163), but this is most likely due to impaired β-adrenergic response and autonomic dysfunction than to insulin signaling defect per se (164, 165). However, postprandial adipose tissue uptake of fatty acids from VLDL-TG is identical and identically suppressed by insulin (because of insulin-mediated reduction in VLDL-TG secretion rate) in patients with T2D versus weight-matched healthy controls (166).

Using administration of deuteriated water with subcutaneous fat biopsies, fractional glycerol synthesis in adipose tissue TG was shown to be reduced in obese subjects with versus without the metabolic syndrome (167). In the latter study, this reduced TG-glycerol synthesis was associated negatively with insulin sensitivity and positively with the plasma NEFA area under the curve (AUC) after an OGTT (167). Likewise, using similar methods, fractional TG-glycerol synthesis and de novo lipogenesis were reduced in insulin-resistant versus sensitive individuals and inversely associated to plasma NEFA levels during fasting and intravenous insulin administration (168). In contrast, fractional TG-glycerol synthesis was higher in adolescent girls with high versus low visceral adipose tissue VAT/total fat mass, in direct proportion with larger VAT/total fat mass or with a higher percent liver fat content (169). It is important to note that the deuterated water method measures new adipose tissue TG synthesis rate from glycerol synthesis and/or de novo lipogenesis, not from the fatty acid esterification rate. Furthermore, because this method requires the administration of deuteriated water over weeks, it is not amenable to experimentally test acute insulin regulation of TG synthesis in adipose tissues in vivo.

Using a positron emission tomography (PET) technique with oral administration of a long-chain fatty acid analog allowing the simultaneous assessment of dietary fatty acid partitioning in most adipose tissues and lean organs (86), we found significant reduction of dietary fat storage *per* volume of abdominal adipose tissues of patients with impaired glucose tolerance (170) that is directly associated with increased waist circumference (171). This reduced density of adipose tissue fat storage is, however, compensated by proportional adipose tissue mass expansion, such that dietary fat partitioning remains on average the same between lean and fat tissues (with the notable exception of elevated partitioning in the myocardium) (161, 171). Furthermore, insulin resistance induced by a 7-day high-saturated fat, high-caloric diet in healthy subjects was actually associated with enhan-ced subcutaneous adipose tissue dietary fatty acid partitioning, reducing the exposure of the heart and skeletal muscles to dietary fat (80). We found that modest weight loss with a 1-year lifestyle intervention increased abdominal adipose tissue dietary fat storage and reduced myocardial dietary fat partitioning in subjects with impaired glucose tolerance (172). Bariatric surgery also led within 12 days to a significant increase in intra-abdominal adipose tissue dietary fatty acid storage and reduction in cardiac partitioning in patients with T2D (79). In the latter study, the increase in intra-abdominal dietary fat storage was associated with the reduction in hepatic insulin resistance after surgery.

In summary, there is clear evidence for impaired postprandial TG storage *per* volume of adipose tissues in insulin-resistant individuals. However, this is likely the result of other factors than insulin resistance per se. Furthermore, adipose tissue mass expansion in general tends to compensate for this impaired density of fat storage. We, however, found that adipose tissue dietary fat storage dynamically changes much faster than fat mass during weight gain or weight loss. More data are needed to understand the role of these adipose tissue metabolic adaptations in the development or remission of insulin resistance and T2D.

Quantitative versus *Qualitative Changes in Plasma NEFA Fluxes in Cardiometabolic Disorders*

The possible causal link between increased NEFA flux from adipose tissues and the development of T2D and complications can be confounded by the heterogeneous lipotoxic effects of the different long-chain fatty acids. Although high plasma NEFA is associated with increased mortality (173), lower circulating polyunsaturated to total fatty acids ratio appears to be an important determinant of that risk (174). Palmitate, the most prevalent circulating saturated fatty acid (~20% of circulating NEFA) displays more diabetogenic effects in vivo in the high physiological and pathophysiological concentration range than oleic or linoleic acid, the most prevalent mono- and polyunsaturated fatty acids in circulation (175). The release of linoleate from peripheral adipose tissues and its partition rate into circulating phospholipids is greater than that of palmitate, underscoring the difference in metabolism and transport of the most prevalent long-chain fatty acids (176–178). With regards to the potential pathogenic role of adipose tissue insulin resistance, it may therefore be preferable to specifically quantify palmitate, not total NEFA flux.

The qualitative versus quantitative differences between meal versus adipose tissue-derived circulating NEFA are also not generally considered when the relationship between adipose tissue insulin resistance and cardiometabolic outcomes is examined. The fatty acid composition of adipose tissues versus plasma is different, with a greater proportion of saturated fatty acids in the latter (179), which is likely the result of the varying relative contribution of meal-derived fatty acids versus intracellular adipose tissue TG lipolysis to Ra_{NEFA} after meals and throughout the day (68, 83). Adipose tissues also have the capacity to elongate and desaturate

palmitate upon excess palmitate uptake or high carbohydrate diets that stimulate de novo lipogenesis (180, 181) and tend to release proportionally more unsaturated fatty acids than palmitate versus their fatty acid content (176). Adipose tissues thus may potentially buffer the lipotoxic effects of the meal-derived saturated fatty acids that are stored and then released through NEFA spillover (182–186). Thus, changes in delivery of dietary fatty acids via NEFA versus direct lipoprotein-derived delivery may modulate the development of lipotoxicity in lean organs. Finally, the main product of de novo lipogenesis is palmitate (187), an important pathophysiological mechanism by which high carbohydrate, high caloric diets lead to hepatic intracellular and VLDL-TG palmitate accumulation and insulin resistance (180, 184, 188–190) and may be associated with increased mortality (191).

In summary, the elucidation of the relationship between adipose tissue insulin resistance and cardiometabolic outcomes will need better integration of dynamic metabolic changes in specific adipose tissue depots and will have to take into account changes in saturated versus unsaturated long-chain fatty acid fluxes from adipose tissue intracellular TG lipolysis, meal NEFA spillover, direct TG-rich lipoprotein delivery to lean organs, and local tissue de novo lipogenesis (Fig. 3).

EFFECT OF INSULIN ON ADIPOSE TISSUE THERMOGENESIS AND ENERGY EXPENDITURE

Over the past decade, the rediscovery of the presence of metabolically active brown adipose tissue (BAT) in humans using PET and other imaging methods has rejuvenated the interest for this very interesting adipose tissue. Insulin plays a major role in the development of this tissue, as illustrated by the BAT-specific insulin receptor + insulin-like growth factor-1 receptor double knockout mouse model, which displays reduced BAT mass and cold-induced thermogenesis, with weight gain and other features of insulin resistance (192). The main energy source for acute cold-induced BAT thermogenesis in humans is its own TG content (193). Acute pharmacological inhibition of intracellular TG lipolysis blunts BAT thermogenesis in rats as well as in humans (194, 195). Therefore, it is plausible that insulin could inhibit BAT intracellular lipolysis, leading to a reduction in cold-induced thermogenesis. In support for this hypothesis, chronic insulin treatment in mice leads to reduced ex vivo respiration and UCP1 and PGC-1α expression in inter-scapular and inguinal, but not in perirenal adipose tissue depots (196). However, Orava et al. (197, 198) found an insulin-mediated increase in BAT glucose uptake in humans, but without change in BAT blood flow, which normally occurs during cold-stimulated BAT thermogenesis. Despite the increase in plasma insulin levels and reduction in plasma NEFA levels and tissue uptake, postprandial BAT and white adipose tissue oxygen consumption was found to be increased by the same group of investigators (199). In mice, cold- or β-adrenergic stimulation lead to the coordinated increase in white and brown adipose tissue lipolysis and insulin secretion (200). In the latter study, inhibition of the adipose tissue lipolytic response prevented the increase in insulin secretion, and the ablation of insulin signaling in BAT blunted the increase in whole-body energy expenditure and BAT fatty acid uptake from lipoproteins. It should be noted that, in contrast to rodents, BAT does not account for a large fraction of NEFA and lipoprotein-derived fatty acid metabolism in humans even after prolonged cold acclimation that increase BAT oxidative metabolism (193, 201, 202). Furthermore, it is unclear at present how much energy expenditure can be accounted for by BAT in humans given the uncertainty related to current methods to measure the metabolically active BAT mass (193).

As discussed in the first section of this review, insulin signaling does not acutely and directly regulate adipocyte respiration in vitro (39, 46). In vivo, insulin or glucose administration are known to reduce the fatty acid oxidation rate by blunting adipose tissue NEFA release and availability to oxidative tissues, but also directly at the mitochondrial level by increasing glucose oxidation and stimulating malonyl-CoA synthesis that inhibits carnitine palmitoyltransferase I activity (203–206). The concomitant increase in glycolysis and glucose oxidation at the whole-body level compensates

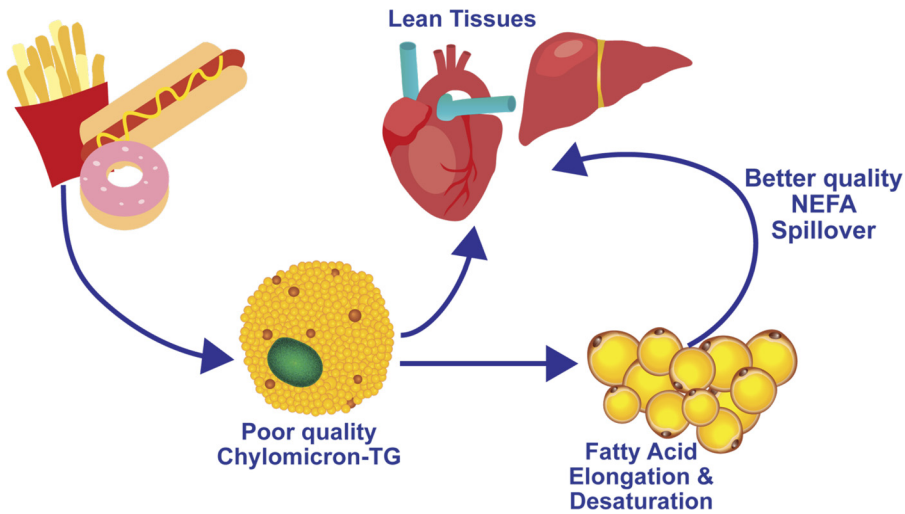

Figure 3. High saturated (poor-quality) dietary fat intake results in poor-quality fatty acid composition of circulating chylomicrons. These poor-quality dietary fatty acids can reach lean tissues directly by hydrolysis of chylomicron-triglycerides and uptake of the fatty acids released in the microcirculation of these tissues or indirectly by first being taken up and rapidly released by adipose tissues (nonesterified fatty acid (NEFA) spillover). The capacity of adipose tissues to elongate and desaturate fatty acids may potentially improve the quality of fatty acids delivered through NEFA spillover *vs.* that of fatty acids delivered directly by chylomicrons. Thus, adipose tissue spillover could be viewed as a protective mechanism against dietary saturated fatty acid lipotoxicity.

for this reduction in fatty acid oxidation, leading to no change in net energy expenditure. We recently found that β3-adrenergic stimulation of whole-body energy expenditure in humans is in large part caused by stimulation of TG/NEFA cycling, not by stimulation of BAT thermogenesis (207). TG/NEFA cycling is, of course, in part regulated by insulin in the postprandial state (see Fig. 1). However, more studies are needed to determine the specific role of insulin in the regulation of adipose tissue energy expenditure through this mechanism in physiological and pathophysiological states in humans.

CONCLUSIONS

Insulin plays a major role in systemic NEFA metabolism both in the fasting and the postprandial states mainly by inhibiting intracellular TG lipolysis and by interrupting the TG lipolysis/NEFA re-esterification cycle in adipose tissues, thus increasing adipose tissue retention of meal-derived fatty acids. Current evidence suggests that insulin stimulation of adipose tissue esterification of fatty acids is increased by hyperglycemic conditions, probably through the stimulation of glycerol-TG synthesis. The concept of "adipose tissue insulin resistance" based on NEFA concentration (or flux) multiplied by insulin level is confounded by many other factors (such as visceral adipose tissue mass, catecholamine signaling defects, etc.) than insulin resistance of adipocyte fatty acid metabolic pathways per se. More studies are needed to better understand the role of insulin and insulin resistance versus these other mechanisms in the regulation of postprandial fat storage, adipose tissue energy expenditure (especially BAT), and lean tissue exposure to potentially toxic fatty acids that plays a role in the development of diabetes and its complications. One hundred years after the discovery of insulin, much is still to be learned about the multifaceted actions of this lifesaving hormone.

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Since 2018, A.C.C. acted as a consultant for Eli Lilly, HLS Therapeutics, Novo Nordisk, Novartis, and Janssen. A.C.C. is the Canada Research Chair in Molecular Imaging of Diabetes.

AUTHOR CONTRIBUTIONS

A.C.C. prepared figures; drafted manuscript; edited and revised manuscript; approved final version of manuscript.

REFERENCES

1. **Roberts J.** Sickening sweet. *Distillations* 1: 12–15, 2015.
2. **Zierler KL**, **Rabinowitz D.** Effect of very small concentrations of insulin on forearm metabolism: persistence of its action on potassium and free fatty acids without its effect on glucose. *J Clin Invest* 43: 950–962, 1964. doi:10.1172/JCI104981.
3. **Miles JM**, **Gerich JE.** Glucose and ketone body kinetics in diabetic ketoacidosis. *Clin Endocrinol Metab* 12: 303–319, 1983. doi:10.1016/S0300-595X(83)80043-7.
4. **Perry RJ**, **Rabin-Court A**, **Song JD**, **Cardone RL**, **Wang Y**, **Kibbey RG**, **Shulman GI.** Dehydration and insulinopenia are necessary and sufficient for euglycemic ketoacidosis in SGLT2 inhibitor-treated rats. *Nat Commun* 10: 548, 2019. doi:10.1038/s41467-019-08466-w.
5. **Voss TS**, **Vendelbo MH**, **Kampmann U**, **Pedersen SB**, **Nielsen TS**, **Johannsen M**, **Svart MV**, **Jessen N**, **Møller N.** Substrate metabolism, hormone and cytokine levels and adipose tissue signalling in individuals with type 1 diabetes after insulin withdrawal and subsequent insulin therapy to model the initiating steps of ketoacidosis. *Diabetologia* 62: 494–503, 2019. doi:10.1007/s00125-018-4785-x.
6. **Giacca A**, **Xiao C**, **Oprescu AI**, **Carpentier AC**, **Lewis GF.** Lipid-induced pancreatic beta-cell dysfunction: focus on in vivo studies. *Am J Physiol Endocrinol Metab* 300: E255–E262, 2011. doi:10.1152/ajpendo.00416.2010.
7. **Lewis GF**, **Carpentier A**, **Adeli K**, **Giacca A.** Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr Rev* 23: 201–229, 2002. doi:10.1210/edrv.23.2.0461.
8. **Samuel VT**, **Petersen KF**, **Shulman GI.** Lipid-induced insulin resistance: unravelling the mechanism. *Lancet* 375: 2267–2277, 2010. doi:10.1016/S0140-6736(10)60408-4.
9. **Unger RH.** Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes* 44: 863–870, 1995. doi:10.2337/diabetes.44.8.863.
10. **Unger RH**, **Clark GO**, **Scherer PE**, **Orci L.** Lipid homeostasis, lipotoxicity and the metabolic syndrome. *Biochim Biophys Acta* 1801: 209–214, 2010. doi:10.1016/j.bbalip.2009.10.006.
11. **Shafrir E**, **Raz I.** Diabetes: mellitus or lipidus? *Diabetologia* 46: 433–440, 2003. doi:10.1007/s00125-003-1052-5.
12. **Frayn KN.** Adipose tissue as a buffer for daily lipid flux. *Diabetologia* 45: 1201–1210, 2002. doi:10.1007/s00125-002-0873-y.
13. **Ofenheimer A**, **Breyer-Kohansal R**, **Hartl S**, **Burghuber OC**, **Krach F**, **Schrott A**, **Wouters EFM**, **Franssen FME**, **Breyer , MK.** Reference values of body composition parameters and visceral adipose tissue (VAT) by DXA in adults aged 18-81 years-results from the LEAD cohort. *Eur J Clin Nutr* 74: 1181–1191, 2020. doi:10.1038/s41430-020-0596-5.
14. **Piche ME**, **Tchernof A**, **Despres JP.** Obesity phenotypes, diabetes, and cardiovascular diseases. *Circ Res* 126: 1477–1500, 2020. [Erratum in *Circ Res*. 127: e107, 2020]. doi:10.1161/CIRCRESAHA.120.316101.
15. **Neeland IJ**, **Ross R**, **Despres JP**, **Matsuzawa Y**, **Yamashita S**, **Shai I**, **Seidell J**, **Magni P**, **Santos RD**, **Arsenault B**, **Cuevas A**, **Hu FB**, **Griffin B**, **Zambon A**, **Barter P**, **Fruchart JC**, **Eckel RH**, **International Atherosclerosis S, and International Chair on Cardiometabolic Risk Working Group on Visceral O.** Visceral and ectopic fat, atherosclerosis, and cardiometabolic disease: a position statement. *Lancet Diabetes Endocrinol* 7: 715–725, 2019. doi:10.1016/S2213-8587(19)30084-1.
16. **Lotta LA**, **Gulati P**, **Day FR**, **Payne F**, **Ongen H**, **van de Bunt M**, **Cambridge FPLD1 Consortium**, et al**.** Integrative genomic analysis implicates limited peripheral adipose storage capacity in the pathogenesis of human insulin resistance. *Nat Genet* 49: 17–26, 2017. doi:10.1038/ng.3714.
17. **Gray SL**, **Vidal-Puig AJ.** Adipose tissue expandability in the maintenance of metabolic homeostasis. *Nutr Rev* 65: S7–12, 2007. doi:10.1111/j.1753-4887.2007.tb00331.x.
18. **Kahn CR**, **Wang G**, **Lee KY.** Altered adipose tissue and adipocyte function in the pathogenesis of metabolic syndrome. *J Clin Invest* 129: 3990–4000, 2019. doi:10.1172/JCI129187.
19. **Bjornson E**, **Adiels M**, **Taskinen MR**, **Borén J.** Kinetics of plasma triglycerides in abdominal obesity. *Curr Opin Lipidol* 28: 11–18, 2017. doi:10.1097/MOL.0000000000000375.
20. **Dash S**, **Xiao C**, **Morgantini C**, **Lewis GF.** New insights into the regulation of chylomicron production. *Annu Rev Nutr* 35: 265–294, 2015. doi:10.1146/annurev-nutr-071714-034338.
21. **Haas ME**, **Attie AD**, **Biddinger SB.** The regulation of ApoB metabolism by insulin. *Trends Endocrinol Metab* 24: 391–397, 2013. doi:10.1016/j.tem.2013.04.001.

22. **Cignarelli A**, **Genchi VA**, **Perrini S**, **Natalicchio A**, **Laviola L**, **Giorgino F.** Insulin and insulin receptors in adipose tissue development. *Int J Mol Sci* 20, 759, 2019. doi:10.3390/ijms20030759.
23. **Marques-Oliveira GH**, **Silva TM**, **Lima WG**, **Valadares HMS**, **Chaves VE.** Insulin as a hormone regulator of the synthesis and release of leptin by white adipose tissue. *Peptides* 106: 49–58, 2018. doi:10.1016/j.peptides.2018.06.007.
24. **Bódis K**, **Roden M.** Energy metabolism of white adipose tissue and insulin resistance in humans. *Eur J Clin Invest* 48: e13017, 2018. doi:10.1111/eci.13017.
25. **Petersen MC**, **Shulman GI.** Mechanisms of insulin action and insulin resistance. *Physiol Rev* 98: 2133–2223, 2018. doi:10.1152/physrev.00063.2017.
26. **Zhao J**, **Wu Y**, **Rong X**, **Zheng C**, **Guo J.** Anti-lipolysis induced by insulin in diverse pathophysiologic conditions of adipose tissue. *Diabetes Metab Syndr Obes* 13: 1575–1585, 2020. doi:10.2147/DMSO.S250699.
27. **Bolinder J**, **Kager L**, **Ostman J**, **Arner P.** Differences at the receptor and postreceptor levels between human omental and subcutaneous adipose tissue in the action of insulin on lipolysis. *Diabetes* 32: 117–123, 1983. doi:10.2337/diabetes.32.2.117.
28. **Van HV**, **Dicker A**, **Ryden M**, **Hauner H**, **Lonnqvist F**, **Naslund E**, **Arner P.** Increased lipolysis and decreased leptin production by human omental as compared with subcutaneous preadipocytes. *Diabetes* 51: 2029–2036, 2002. doi:10.2337/diabetes.51.7.2029.
29. **Zierath JR**, **Livingston JN**, **Thorne A**, **Bolinder J**, **Reynisdottir S**, **Lonnqvist F**, **Arner P.** Regional difference in insulin inhibition of nonesterified fatty acid release from human adipocytes: relation to insulin receptor phosphorylation and intracellular signalling through the insulin receptor substrate-1 pathway. *Diabetologia* 41: 1343–1354, 1998. doi:10.1007/s001250051075.
30. **Arner P**, **Bolinder J**, **Engfeldt P**, **Hellmér J**, **Ostman J.** Influence of obesity on the antilipolytic effect of insulin in isolated human fat cells obtained before and after glucose ingestion. *J Clin Invest* 73: 673–680, 1984. doi:10.1172/JCI111259.
31. **Arner P**, **Bolinder J**, **Engfeldt P**, **Ostman J.** The antilipolytic effect of insulin in human adipose tissue in obesity, diabetes mellitus, hyperinsulinemia, and starvation. *Metab Clin Exp* 30: 753–760, 1981. doi:10.1016/0026-0495(81)90020-2.
32. **Bolinder J**, **Lithell H**, **Skarfors E**, **Arner P.** Effects of obesity, hyperinsulinemia, and glucose intolerance on insulin action in adipose tissue of sixty-year-old men. *Diabetes* 35: 282–290, 1986. doi:10.2337/diab.35.3.282.
33. **Arner P**, **Andersson DP**, **Backdahl J**, **Dahlman I**, **Rydén M.** Weight gain and impaired glucose metabolism in women are predicted by inefficient subcutaneous fat cell lipolysis. *Cell Metab* 28: 45–54 e43, 2018. doi:10.1016/j.cmet.2018.05.004.
34. **Gao H**, **Arner P**, **Beauchef G**, **Guere C**, **Vie K**, **Dahlman I**, **Mejhert N**, **Rydén M.** Age-induced reduction in human lipolysis: a potential role for adipocyte noradrenaline degradation. *Cell Metab* 32: 1–3, 2020. doi:10.1016/j.cmet.2020.06.007.
35. **Campbell PJ**, **Carlson MG**, **Hill JO**, **Nurjhan N.** Regulation of free fatty acid metabolism by insulin in humans: role of lipolysis and reesterification. *Am J Physiol Endocrinol Physiol* 263: E1063–E1069, 1992. doi:10.1152/ajpendo.2006.263.6.E1063.
36. **Cadoudal T**, **Leroyer S**, **Reis AF**, **Tordjman J**, **Durant S**, **Fouque F**, **Collinet M**, **Quette J**, **Chauvet G**, **Beale E**, **Velho G**, **Antoine B**, **Benelli C**, **Forest C.** Proposed involvement of adipocyte glyceroneogenesis and phosphoenolpyruvate carboxykinase in the metabolic syndrome. *Biochimie* 87: 27–32, 2005. doi:10.1016/j.biochi.2004.12.005.
37. **Meegalla RL**, **Billheimer JT**, **Cheng D.** Concerted elevation of acyl-coenzyme A:diacylglycerol acyltransferase (DGAT) activity through independent stimulation of mRNA expression of DGAT1 and DGAT2 by carbohydrate and insulin. *Biochem Biophys Res Commun* 298: 317–323, 2002. doi:10.1016/S0006-291X(02)02466-X.
38. **Roncari DA**, **Mack EY**, **Yip DK.** Enhancement of microsomal phosphatidate phosphohydrolase and diacylglycerol acyltransferase activity by insulin during growth of rat adipocyte precursors in culture. *Can J Biochem* 57: 573–577, 1979. doi:10.1139/o79-072.
39. **Krycer JR**, **Quek LE**, **Francis D**, **Zadoorian A**, **Weiss FC**, **Cooke KC**, **Nelson ME**, **Diaz-Vegas A**, **Humphrey SJ**, **Scalzo R**, **Hirayama A**, **Ikeda S**, **Shoji F**, **Suzuki K**, **Huynh K**, **Giles C**, **Varney B**, **Nagarajan SR**, **Hoy AJ**, **Soga T**, **Meikle PJ**, **Cooney GJ**, **Fazakerley DJ**, **James DE.** Insulin signaling requires glucose to promote lipid anabolism in adipocytes. *J Biol Chem* 295: 13250–13266, 2020. doi:10.1074/jbc.RA120.014907.
40. **May JM.** Triacylglycerol turnover in large and small rat adipocytes: effects of lipolytic stimulation, glucose, and insulin. *J Lipid Res* 23: 428–436, 1982. doi:10.1016/S0022-2275(20)38139-6.
41. **Tozzo E**, **Shepherd PR**, **Gnudi L**, **Kahn BB.** Transgenic GLUT-4 overexpression in fat enhances glucose metabolism: preferential effect on fatty acid synthesis. *Am J Physiol Endocrinol Metab* 268: E956–E964, 1995.
42. **Wong RH**, **Sul HS.** Insulin signaling in fatty acid and fat synthesis: a transcriptional perspective. *Curr Opin Pharmacol* 10: 684–691, 2010. doi:10.1016/j.coph.2010.08.004.
43. **Ranganathan G**, **Unal R**, **Pokrovskaya I**, **Yao-Borengasser A**, **Phanavanh B**, **Lecka-Czernik B**, **Rasouli N**, **Kern PA.** The lipogenic enzymes DGAT1, FAS, and LPL in adipose tissue: effects of obesity, insulin resistance, and TZD treatment. *J Lipid Res* 47: 2444–2450, 2006. doi:10.1194/jlr.M600248-JLR200.
44. **Bohm A**, **Keuper M**, **Meile T**, **Zdichavsky M**, **Fritsche A**, **Häring HU**, **de Angelis MH**, **Staiger H**, **Franko A.** Increased mitochondrial respiration of adipocytes from metabolically unhealthy obese compared to healthy obese individuals. *Sci Rep* 10: 12407, 2020. doi:10.1038/s41598-020-69016-9.
45. **Politis-Barber V**, **Brunetta HS**, **Paglialunga S**, **Petrick HL**, **Holloway GP.** Long-term, high-fat feeding exacerbates short-term increases in adipose mitochondrial reactive oxygen species, without impairing mitochondrial respiration. *Am J Physiol Endocrinol Metab* 319: E376–E387, 2020. doi:10.1152/ajpendo.00028.2020.
46. **Olsen JM**, **Aslund A**, **Bokhari MH**, **Hutchinson DS**, **Bengtsson T.** Acute β-adrenoceptor mediated glucose clearance in brown adipose tissue; a distinct pathway independent of functional insulin signaling. *Mol Metab* 30: 240–249, 2019. doi:10.1016/j.molmet.2019.10.004.
47. **Søndergaard E**, **Espinosa De Ycaza AE**, **Morgan-Bathke M**, **Jensen MD.** How to measure adipose tissue insulin sensitivity. *J Clin Endocrinol Metab* 102: 1193–1199, 2017. doi:10.1210/jc.2017-00047.
48. **Reshef L**, **Olswang Y**, **Cassuto H**, **Blum B**, **Croniger CM**, **Kalhan SC**, **Tilghman SM**, **Hanson RW.** Glyceroneogenesis and the triglyceride/fatty acid cycle. *J Biol Chem* 278: 30413–30416, 2003. doi:10.1074/jbc.R300017200.
49. **Nurjhan N**, **Kennedy F**, **Consoli A**, **Martin C**, **Miles J**, **Gerich J.** Quantification of the glycolytic origin of plasma glycerol: implications for the use of the rate of appearance of plasma glycerol as an index of lipolysis in vivo. *Metab Clin Exp* 37: 386–389, 1988. doi:10.1016/0026-0495(88)90140-0.
50. **Carpentier AC**, **Frisch F**, **Brassard P**, **Lavoie F**, **Bourbonnais A**, **Cyr D**, **Giguère R**, **Baillargeon JP.** Mechanism of insulin-stimulated clearance of plasma nonesterified fatty acids in humans. *Am J Physiol Endocrinol Metab* 292: E693–E701, 2007. doi:10.1152/ajpendo.00423.2006.
51. **Normand-Lauzière F**, **Frisch F**, **Labbé SM**, **Bherer P**, **Gagnon R**, **Cunnane SC**, **Carpentier AC.** Increased postprandial nonesterified fatty acid appearance and oxidation in type 2 diabetes is not fully established in offspring of diabetic subjects. *PLoS ONE* 5: e10956, 2010. doi:10.1371/journal.pone.0010956.
52. **Hammond VA**, **Johnston DG.** Substrate cycling between triglyceride and fatty acid in human adipocytes. *Metab Clin Exp* 36: 308–313, 1987. doi:10.1016/0026-0495(87)90199-5.
53. **Leibel RL**, **Forse RA**, **Hirsch J.** Effects of rapid glucose infusion on in vivo and in vitro free fatty acid re-esterification by adipose tissue of fasted obese subjects. *Int J Obes* 13: 661–671, 1989.
54. **Wolfe RR**, **Peters EJ.** Lipolytic response to glucose infusion in human subjects. *Am J Physiol Endocrinol Metab* 252: E218–E223, 1987. doi:10.1152/ajpendo.1987.252.2.E218.
55. **Coppack SW**, **Frayn KN**, **Humphreys SM**, **Dhar H**, **Hockaday TD.** Effects of insulin on human adipose tissue metabolism in vivo. *Clin Sci (Lond)* 77: 663–670, 1989. doi:10.1042/cs0770663.
56. **Ali AH**, **Mundi M**, **Koutsari C**, **Bernlohr DA**, **Jensen MD.** Adipose tissue free fatty acid storage in vivo: effects of insulin versus niacin as a control for suppression of lipolysis. *Diabetes* 64: 2828–2835, 2015. doi:10.2337/db14-1409. doi:10.2337/db14-1409.
57. **Bickerton AS**, **Roberts R**, **Fielding BA**, **Hodson L**, **Blaak EE**, **Wagenmakers AJ**, **Gilbert M**, **Karpe F**, **Frayn KN.** Preferential uptake of dietary Fatty acids in adipose tissue and muscle in the

postprandial period. *Diabetes* 56: 168–176, 2007. doi:10.2337/db06-0822.
58. **Carpentier AC**, **Labbe SM**, **Grenier-Larouche T**, **Noll C.** Abnormal dietary fatty acid metabolic partitioning in insulin resistance and type 2 diabetes. *Clin Lipidol* 6: 703–716, 2011. doi:10.2217/clp.11.60.
59. **Miles JM**, **Park YS**, **Walewicz D**, **Russell-Lopez C**, **Windsor S**, **Isley WL**, **Coppack SW**, **Harris WS.** Systemic and forearm triglyceride metabolism: fate of lipoprotein lipase-generated glycerol and free fatty acids. *Diabetes* 53: 521–527, 2004. doi:10.2337/diabetes.53.3.521.
60. **Bush NC**, **Triay JM**, **Gathaiya NW**, **Hames KC**, **Jensen MD.** Contribution of very low-density lipoprotein triglyceride fatty acids to postabsorptive free fatty acid flux in obese humans. *Metabolism* 63: 137–140, 2014. doi:10.1016/j.metabol.2013.09.008.
61. **Nelson RH**, **Basu R**, **Johnson CM**, **Rizza RA**, **Miles JM.** Splanchnic spillover of extracellular lipase-generated fatty acids in overweight and obese humans. *Diabetes* 56: 2878–2884, 2007. doi:10.2337/db07-0812.
62. **Nelson RH**, **Edgerton DS**, **Basu R**, **Roesner JC**, **Cherrington AD**, **Miles JM.** Triglyceride uptake and lipoprotein lipase-generated fatty acid spillover in the splanchnic bed of dogs. *Diabetes* 56: 1850–1855, 2007. doi:10.2337/db06-1657.
63. **Nelson RH**, **Prasad A**, **Lerman A**, **Miles JM.** Myocardial uptake of circulating triglycerides in nondiabetic patients with heart disease. *Diabetes* 56: 527–530, 2007. doi:10.2337/db06-1552.
64. **Roust LR**, **Jensen MD.** Postprandial free fatty acid kinetics are abnormal in upper body obesity. *Diabetes* 42: 1567–1573, 1993. doi:10.2337/diab.42.11.1567.
65. **Barrows BR**, **Timlin MT**, **Parks EJ.** Spillover of dietary fatty acids and use of serum nonesterified fatty acids for the synthesis of VLDL-triacylglycerol under two different feeding regimens. *Diabetes* 54: 2668–2673, 2005. doi:10.2337/diabetes.54.9.2668.
66. **Timlin MT**, **Barrows BR**, **Parks EJ.** Increased dietary substrate delivery alters hepatic fatty acid recycling in healthy men. *Diabetes* 54: 2694–2701, 2005. doi:10.2337/diabetes.54.9.2694.
67. **Tan GD**, **Fielding BA**, **Currie JM**, **Humphreys SM**, **Désage M**, **Frayn KN**, **Laville M**, **Vidal H**, **Karpe F.** The effects of rosiglitazone on fatty acid and triglyceride metabolism in type 2 diabetes. *Diabetologia* 48: 83–95, 2005. doi:10.1007/s00125-004-1619-9.
68. **Ruge T**, **Hodson L**, **Cheeseman J**, **Dennis AL**, **Fielding BA**, **Humphreys SM**, **Frayn KN**, **Karpe F.** Fasted to fed trafficking of fatty acids in human adipose tissue reveals a novel regulatory step for enhanced fat storage. *J Clin Endocrinol Metab* 94: 1781–1788, 2009. doi:10.1210/jc.2008-2090.
69. **Nelson RH**, **Vlazny D**, **Smailovic A**, **Miles JM.** Intravenous niacin acutely improves the efficiency of dietary fat storage in lean and obese humans. *Diabetes* 61: 3172–3175, 2012. doi:10.2337/db12-0236.
70. **Muthusamy K**, **Nelson RH**, **Singh E**, **Vlazny D**, **Smailovic A**, **Miles JM.** Effect of insulin infusion on spillover of meal-derived fatty acids. *J Clin Endocrinol Metab* 97: 4201–4205, 2012. doi:10.1210/jc.2012-1468.
71. **Puga GM**, **Meyer C**, **Mandarino LJ**, **Katsanos CS.** Postprandial spillover of dietary lipid into plasma is increased with moderate amounts of ingested fat and is inversely related to adiposity in healthy older men. *J Nutr* 142: 1806–1811, 2012. doi:10.3945/jn.112.162008.
72. **Alligier M**, **Gabert L**, **Meugnier E**, **Lambert-Porcheron S**, **Chanseaume E**, **Pilleul F**, **Debard C**, **Sauvinet V**, **Morio B**, **Vidal-Puig A**, **Vidal H**, **Laville M.** Visceral fat accumulation during lipid overfeeding is related to subcutaneous adipose tissue characteristics in healthy men. *J Clin Endocrinol Metab* 98: 802–810, 2013. doi:10.1210/jc.2012-3289.
73. **Almandoz JP**, **Singh E**, **Howell LA**, **Grothe K**, **Vlazny DT**, **Smailovic A**, **Irving BA**, **Nelson RH**, **Miles JM.** Spillover of fatty acids during dietary fat storage in type 2 diabetes: relationship to body fat depots and effects of weight loss. *Diabetes* 62: 1897–1903, 2013. doi:10.2337/db12-1407.
74. **Nelson RH**, **Mundi MS**, **Vlazny DT**, **Smailovic A**, **Muthusamy K**, **Almandoz JP**, **Singh E**, **Jensen MD**, **Miles JM.** Kinetics of saturated, monounsaturated, and polyunsaturated fatty acids in humans. *Diabetes* 62: 783–788, 2013. doi:10.2337/db12-0367.
75. **Vors C**, **Pineau G**, **Gabert L**, **Drai J**, **Louche-Pelissier C**, **Defoort C**, **Lairon D**, **Désage M**, **Danthine S**, **Lambert-Porcheron S**, **Vidal H**, **Laville M**, **Michalski MC.** Modulating absorption and postprandial handling of dietary fatty acids by structuring fat in the meal: a randomized crossover clinical trial. *Am J Clin Nutr* 97: 23–36, 2013. doi:10.3945/ajcn.112.043976.
76. **Noll C**, **Kunach M**, **Frisch F**, **Bouffard L**, **Dubreuil S**, **Jean-Denis F**, **Phoenix S**, **Cunnane SC**, **Guérin B**, **Turcotte EE**, **Carpentier AC.** Seven-day caloric and saturated fat restriction increases myocardial dietary fatty acid partitioning in impaired glucose-tolerant subjects. *Diabetes* 64: 3690–3699, 2015. doi:10.2337/db15-0337.
77. **Grenier-Larouche T**, **Carreau AM**, **Geloën A**, **Frisch F**, **Biertho L**, **Marceau S**, **Lebel S**, **Hould FS**, **Richard D**, **Tchernof A**, **Carpentier AC.** Fatty acid metabolic remodeling during type 2 diabetes remission after bariatric surgery. *Diabetes* 66: 2743–2755, 2017. doi:10.2337/db17-0414.
78. **Piche ME**, **Parry SA**, **Karpe F**, **Hodson L.** Chylomicron-derived fatty acid spillover in adipose tissue: a signature of metabolic health? *J Clin Endocrinol Metab* 103: 25–34, 2018. doi:10.1210/jc.2017-01517.
79. **Carreau AM**, **Noll C**, **Blondin DP**, **Frisch F**, **Nadeau M**, **Pelletier M**, **Phoenix S**, **Cunnane SC**, **Guérin B**, **Turcotte EE**, **Lebel S**, **Biertho L**, **Tchernof A**, **Carpentier AC.** Bariatric surgery rapidly decreases cardiac dietary fatty acid partitioning and hepatic insulin resistance through increased intra-abdominal adipose tissue storage and reduced spillover in type 2 diabetes. *Diabetes* 69: 567–577, 2020. doi:10.2337/db19-0773.
80. **Noll C**, **Montastier E**, **Amrani M**, **Kunach M**, **Frisch F**, **Fortin M**, **Bouffard L**, **Dubreuil S**, **Phoenix S**, **Cunnane SC**, **Guérin B**, **Turcotte EE**, **Laville M**, **Carpentier AC.** Seven-day overfeeding enhances adipose tissue dietary fatty acid storage and decreases myocardial and skeletal muscle dietary fatty acid partitioning in healthy subjects. *Am J Physiol Endocrinol Metab* 318: E286–E296, 2020. doi:10.1152/ajpendo.00474.2019.
81. **Carpentier A**, **Frisch F**, **Cyr D**, **Genereux P**, **Patterson BW**, **Giguère R**, **Baillargeon JP.** On the suppression of plasma non-esterified fatty acids by insulin during enhanced intravascular lipolysis in humans. *Am J Physiol Endocrinol Metab* 289: E849–E856, 2005. doi:10.1152/ajpendo.00073.2005.
82. **Frayn KN**, **Shadid S**, **Hamlani R**, **Humphreys SM**, **Clark ML**, **Fielding BA**, **Boland O**, **Coppack SW.** Regulation of fatty acid movement in human adipose tissue in the postabsorptive-to-postprandial transition. *Am J Physiol Endocrinol Metab* 266: E308–E317, 1994. doi:10.1152/ajpendo.1994.266.3.E308.
83. **Evans K**, **Burdge GC**, **Wootton SA**, **Clark ML**, **Frayn KN.** Regulation of dietary Fatty Acid entrapment in subcutaneous adipose tissue and skeletal muscle. *Diabetes* 51: 2684–2690, 2002. doi:10.2337/diabetes.51.9.2684.
84. **Summers LK**, **Barnes SC**, **Fielding BA**, **Beysen C**, **Ilic V**, **Humphreys SM**, **Frayn KN.** Uptake of individual fatty acids into adipose tissue in relation to their presence in the diet. *Am J Clin Nutr* 71: 1470–1477, 2000. doi:10.1093/ajcn/71.6.1470.
85. **Jensen MD**, **Sarr MG**, **Dumesic DA**, **Southorn PA**, **Levine JA.** Regional uptake of meal fatty acids in humans. *Am J Physiol Endocrinol Metab* 285: E1282–E1288, 2003. doi:10.1152/ajpendo.00220.2003.
86. **Labbé SM**, **Grenier-Larouche T**, **Croteau E**, **Normand-Lauzière F**, **Frisch F**, **Ouellet R**, **Guérin B**, **Turcotte EE**, **Carpentier AC.** Organ-specific dietary fatty acid uptake in humans using positron emission tomography coupled to computed tomography. *Am J Physiol Endocrinol Metab* 300: E445–E453, 2011. doi:10.1152/ajpendo.00579.2010.
87. **Marin P**, **Lonn L**, **Andersson B**, **Oden B**, **Olbe L**, **Bengtsson BA**, **Bjorntorp P.** Assimilation of triglycerides in subcutaneous and intraabdominal adipose tissues in vivo in men: effects of testosterone. *J Clin Endocrinol Metab* 81: 1018–1022, 1996. doi:10.1210/jcem.81.3.8772568.
88. **Votruba SB**, **Mattison RS**, **Dumesic DA**, **Koutsari C**, **Jensen MD.** Meal fatty acid uptake in visceral fat in women. *Diabetes* 56: 2589–2597, 2007. doi:10.2337/db07-0439.
89. **Fielding BA**, **Frayn KN.** Lipoprotein lipase and the disposition of dietary fatty acids. *Br J Nutr* 80: 495–502, 1998. doi:10.1017/S0007114598001585.
90. **Coppack SW**, **Frayn KN**, **Humphreys SM.** Plasma triacylglycerol extraction in human adipose tissue in vivo: effects of glucose ingestion and insulin infusion. *Eur J Clin Nutr* 43: 493–496, 1989.
91. **Vatner DF**, **Majumdar SK**, **Kumashiro N**, **Petersen MC**, **Rahimi Y**, **Gattu AK**, **Bears M**, **Camporez JP**, **Cline GW**, **Jurczak MJ**, **Samuel VT**, **Shulman GI.** Insulin-independent regulation of hepatic

triglyceride synthesis by fatty acids. *Proc Natl Acad Sci USA* 112: 1143–1148, 2015. doi:10.1073/pnas.1423952112.
92. **Bülow J**, **Simonsen L**, **Wiggins D**, **Humphreys SM**, **Frayn KN**, **Powell D**, **Gibbons GF.** Co-ordination of hepatic and adipose tissue lipid metabolism after oral glucose. *J Lipid Res* 40: 2034–2043, 1999. doi:10.1016/S0022-2275(20)32427-5.
93. **Evans K**, **Clark ML**, **Frayn KN.** Effects of an oral and intravenous fat load on adipose tissue and forearm lipid metabolism. *Am J Physiol Endocrinol Metab* 276: E241–E248, 1999. doi:10.1152/ajpendo.1999.276.2.E241.
94. **Samra JS**, **Giles SL**, **Summers LK**, **Evans RD**, **Arner P**, **Humphreys SM**, **Clark ML**, **Frayn KN.** Peripheral fat metabolism during infusion of an exogenous triacylglycerol emulsion. *Int J Obes Relat Metab Disord* 22: 806–812, 1998. doi:10.1038/sj.ijo.0800664.
95. **Karpe F**, **Fielding BA**, **Ardilouze JL**, **Ilic V**, **Macdonald IA**, **Frayn KN.** Effects of insulin on adipose tissue blood flow in man. *J Physiol* 540: 1087–1093, 2002. doi:10.1113/jphysiol.2001.013358.
96. **Votruba SB**, **Jensen MD.** Sex-specific differences in leg fat uptake are revealed with a high-fat meal. *Am J Physiol Endocrinol Metab* 291: E1115–E1123, 2006. doi:10.1152/ajpendo.00196.2006.
97. **Santosa S**, **Hensrud DD**, **Votruba SB**, **Jensen MD.** The influence of sex and obesity phenotype on meal fatty acid metabolism before and after weight loss. *Am J Clin Nutr* 88: 1134–1141, 2008. doi:10.1093/ajcn/88.4.1134.
98. **Santosa S**, **Bush NC**, **Jensen MD.** Acute testosterone deficiency alters adipose tissue fatty acid storage. *J Clin Endocrinol Metab* 102: 3056–3064, 2017. doi:10.1210/jc.2017-00757.
99. **Asmar M**, **Asmar A**, **Simonsen L**, **Gasbjerg LS**, **Sparre-Ulrich AH**, **Rosenkilde MM**, **Hartmann B**, **Dela F**, **Holst JJ**, **Bülow J.** The gluco- and liporegulatory and vasodilatory effects of glucose-dependent insulinotropic polypeptide (GIP) are abolished by an antagonist of the human GIP receptor. *Diabetes* 66: 2363–2371, 2017. doi:10.2337/db17-0480.
100. **Bonen A**, **Campbell SE**, **Benton CR**, **Chabowski A**, **Coort SL**, **Han XX**, **Koonen DP**, **Glatz JF**, **Luiken JJ.** Regulation of fatty acid transport by fatty acid translocase/CD36. *Proc Nutr Soc* 63: 245–249, 2004. doi:10.1079/PNS2004331.
101. **Chabowski A**, **Coort SL**, **Calles-Escandon J**, **Tandon NN**, **Glatz JF**, **Luiken JJ**, **Bonen A.** Insulin stimulates fatty acid transport by regulating the expression of FAT/CD36 but not FABPpm. *Am J Physiol Endocrinol Metab* 287: E781–E789, 2004. doi:10.1152/ajpendo.00573.2003.
102. **Miles JM**, **Wooldridge D**, **Grellner WJ**, **Windsor S**, **Isley WL**, **Klein S**, **Harris WS.** Nocturnal and postprandial free fatty acid kinetics in normal and type 2 diabetic subjects: effects of insulin sensitization therapy. *Diabetes* 52: 675–681, 2003. doi:10.2337/diabetes.52.3.675.
103. **Mittendorfer B**, **Liem O**, **Patterson BW**, **Miles JM**, **Klein S.** What does the measurement of whole-body fatty acid rate of appearance in plasma by using a fatty acid tracer really mean? *Diabetes* 52: 1641–1648, 2003. doi:10.2337/diabetes.52.7.1641.
104. **Ramos-Roman MA**, **Lapidot SA**, **Phair RD**, **Parks EJ.** Insulin activation of plasma nonesterified fatty acid uptake in metabolic syndrome. *Arterioscler Thromb Vasc Biol* 32: 1799–1808, 2012. doi:10.1161/ATVBAHA.112.250019.
105. **Furler SM**, **Cooney GJ**, **Hegarty BD**, **Lim-Fraser MY**, **Kraegen EW**, **Oakes ND.** Local factors modulate tissue-specific NEFA utilization: assessment in rats using ^{3}H-(R)-2-bromopalmitate. *Diabetes* 49: 1427–1433, 2000. doi:10.2337/diabetes.49.9.1427.
106. **Ci X**, **Frisch F**, **Lavoie F**, **Germain P**, **Lecomte R**, **van Lier JE**, **Bénard F**, **Carpentier AC.** The effect of insulin on the intracellular distribution of 14(R,S)-[(18)F]Fluoro-6-thia-heptadecanoic acid in rats. *Mol Imaging Biol* 8: 237–244, 2006. doi:10.1007/s11307-006-0042-7.
107. **Guzzardi MA**, **Hodson L**, **Guiducci L**, **La Rosa F**, **Salvadori PA**, **Burchielli S**, **Iozzo P.** The role of glucose, insulin and NEFA in regulating tissue triglyceride accumulation: Substrate cooperation in adipose tissue versus substrate competition in skeletal muscle. *Nutr Metab Cardiovasc Dis* 27: 956–963, 2017. doi:10.1016/j.numecd.2017.08.002.
108. **Ter Horst KW**, **van Galen KA**, **Gilijamse PW**, **Hartstra AV**, **de Groot PF**, **van der Valk FM**, **Ackermans MT**, **Nieuwdorp M**, **Romijn JA**, **Serlie MJ.** Methods for quantifying adipose tissue insulin resistance in overweight/obese humans. *Int J Obes* 41: 1288–1294, 2017. doi:10.1038/ijo.2017.110.
109. **Johansson E**, **Lubberink M**, **Heurling K**, **Eriksson JW**, **Skrtic S**, **Ahlström H**, **Kullberg J.** Whole-body imaging of tissue-specific insulin sensitivity and body composition by using an integrated PET/MR System: a feasibility study. *Radiology* 286: 271–278, 2018. doi:10.1148/radiol.2017162949.
110. **Boersma GJ**, **Johansson E**, **Pereira MJ**, **Heurling K**, **Skrtic S**, **Lau J**, **Katsogiannos P**, **Panagiotou G**, **Lubberink M**, **Kullberg J**, **Ahlström H**, **Eriksson JW.** Altered glucose uptake in muscle, visceral adipose tissue, and brain predict whole-body insulin resistance and may contribute to the development of type 2 diabetes: a combined PET/MR study. *Horm Metab Res* 50: 627–639, 2018. doi:10.1055/a-0643-4739.
111. **Rached MT**, **Millership SJ**, **Pedroni SMA**, **Choudhury AI**, **Costa ASH**, **Hardy DG**, **Glegola JA**, **Irvine EE**, **Selman C**, **Woodberry MC**, **Yadav VK**, **Khadayate S**, **Vidal-Puig A**, **Virtue S**, **Frezza C**, **Withers DJ.** Deletion of myeloid IRS2 enhances adipose tissue sympathetic nerve function and limits obesity. *Mol Metab* 20: 38–50, 2019. doi:10.1016/j.molmet.2018.11.010.
112. **Toda G**, **Soeda K**, **Okazaki Y**, **Kobayashi N**, **Masuda Y**, **Arakawa N**, **Suwanai H**, **Masamoto Y**, **Izumida Y**, **Kamei N**, **Sasako T**, **Suzuki R**, **Kubota T**, **Kubota N**, **Kurokawa M**, **Tobe K**, **Noda T**, **Honda K**, **Accili D**, **Yamauchi T**, **Kadowaki T**, **Ueki K.** Insulin- and lipopolysaccharide-mediated signaling in adipose tissue macrophages regulates postprandial glycemia through Akt-mTOR activation. *Mol Cell* 79: 43–53.e4, 2020. doi:10.1016/j.molcel.2020.04.033.
113. **Wang C**, **Li X**, **Dang H**, **Liu P**, **Zhang BO**, **Xu F.** Insulin-like growth factor 2 regulates the proliferation and differentiation of rat adipose-derived stromal cells via IGF-1R and IR. *Cytotherapy* 21: 619–630, 2019. doi:10.1016/j.jcyt.2018.11.010.
114. **Carpentier AC.** Abnormal myocardial dietary fatty acid metabolism and diabetic cardiomyopathy. *Can J Cardiol* 34: 605–614, 2018. doi:10.1016/j.cjca.2017.12.029.
115. **Kenny HC**, **Abel ED.** Heart failure in type 2 diabetes mellitus. *Circ Res* 124: 121–141, 2019. doi:10.1161/CIRCRESAHA.118.311371.
116. **Meex RCR**, **Blaak EE**, **van Loon LJC.** Lipotoxicity plays a key role in the development of both insulin resistance and muscle atrophy in patients with type 2 diabetes. *Obes Rev* 20: 1205–1217, 2019. doi:10.1111/obr.12862.
117. **Opazo-Rios L**, **Mas S**, **Marin-Royo G**, **Mezzano S**, **Gomez-Guerrero C**, **Moreno JA**, **Egido J.** Lipotoxicity and diabetic nephropathy: novel mechanistic insights and therapeutic opportunities. *Int J Mol Sci* 21, 2632, 2020. doi:10.3390/ijms21072632.
118. **Gastaldelli A**, **Gaggini M**, **DeFronzo RA.** Role of adipose tissue insulin resistance in the natural history of type 2 diabetes: results from the San Antonio Metabolism Study. *Diabetes* 66: 815–822, 2017. doi:10.2337/db16-1167.
119. **Hershkop K**, **Besor O**, **Santoro N**, **Pierpont B**, **Caprio S**, **Weiss R.** Adipose insulin resistance in obese adolescents across the spectrum of glucose tolerance. *J Clin Endocrinol Metab* 101: 2423–2431, 2016. doi:10.1210/jc.2016-1376.
120. **Rydén M**, **Arner P.** Cardiovascular risk score is linked to subcutaneous adipocyte size and lipid metabolism. *J Intern Med* 282: 220–228, 2017. doi:10.1111/joim.12641.
121. **Kahn SE**, **Prigeon RL**, **McCulloch DK**, **Boyko EJ**, **Bergman RN**, **Schwartz MW**, **Neifing JL**, **Ward WK**, **Beard JC**, **Palmer JP**, **Porte D. Jr.** Quantification of the relationship between insulin sensitivity and ·-cell function in human subjects: evidence for a hyperbolic function**.** *Diabetes* 42: 1663–1672, 1993. doi:10.2337/diab.42.11.1663.
122. **Malin SK**, **Kashyap SR**, **Hammel J**, **Miyazaki Y**, **DeFronzo RA**, **Kirwan JP.** Adjusting glucose-stimulated insulin secretion for adipose insulin resistance: an index of beta-cell function in obese adults. *Diabetes Care* 37: 2940–2946, 2014. doi:10.2337/dc13-3011.
123. **Gastaldelli A**, **Ghani MA**, **DeFronzo RA.** Adaptation of insulin clearance to metabolic demand is a key determinant of glucose tolerance. *Diabetes* 70: 377–385, 2021. doi:10.2337/db19-1152.
124. **Gastaldelli A**, **Basta G.** Ectopic fat and cardiovascular disease: what is the link? *Nutr Metab Cardiovasc Dis* 20: 481–490, 2010. doi:10.1016/j.numecd.2010.05.005.
125. **Lee JJ**, **Pedley A**, **Hoffmann U**, **Massaro JM**, **Levy D**, **Long MT.** Visceral and intrahepatic fat are associated with cardiometabolic risk factors above other ectopic fat depots: the Framingham Heart Study. *Am J Med* 131: 684–692.e12, 2018. doi:10.1016/j.amjmed.2018.02.002.

126. **Rawshani A**, **Eliasson B**, **Rawshani A**, **Henninger J**, **Mardinoglu A**, **Carlsson A**, **Sohlin M**, **Ljungberg M**, **Hammarstedt A**, **Rosengren A**, **Smith U.** Adipose tissue morphology, imaging and metabolomics predicting cardiometabolic risk and family history of type 2 diabetes in non-obese men. *Sci Rep* 10: 9973, 2020. doi:10.1038/s41598-020-66199-z.
127. **Rospleszcz S**, **Lorbeer R**, **Storz C**, **Schlett CL**, **Meisinger C**, **Thorand B**, **Rathmann W**, **Bamberg F**, **Lieb W**, **Peters A.** Association of longitudinal risk profile trajectory clusters with adipose tissue depots measured by magnetic resonance imaging. *Sci Rep* 9: 16972, 2019. doi:10.1038/s41598-019-53546-y.
128. **Barrows BR**, **Parks EJ.** Contributions of different fatty acid sources to very low-density lipoprotein-triacylglycerol in the fasted and fed states. *J Clin Endocrinol Metab* 91: 1446–1452, 2006. doi:10.1210/jc.2005-1709.
129. **Donnelly KL**, **Smith CI**, **Schwarzenberg SJ**, **Jessurun J**, **Boldt MD**, **Parks EJ.** Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest* 115: 1343–1351, 2005. doi:10.1172/JCI23621.
130. **Parks EJ**, **Krauss RM**, **Christiansen MP**, **Neese RA**, **Hellerstein MK.** Effects of a low-fat, high-carbohydrate diet on VLDL-triglyceride assembly, production, and clearance. *J Clin Invest* 104: 1087–1096, 1999. doi:10.1172/JCI6572.
131. **Barchetta I**, **Angelico F**, **Del Ben M**, **Di Martino M**, **Cimini FA**, **Bertoccini L**, **Polimeni L**, **Catalano C**, **Fraioli A**, **Del Vescovo R**, **Morini S**, **Baroni MG**, **Cavallo MG.** Phenotypical heterogeneity linked to adipose tissue dysfunction in patients with Type 2 diabetes. *Clin Sci (Lond)* 130: 1753–1762, 2016. doi:10.1042/CS20160348.
132. **Bril F**, **Barb D**, **Portillo-Sanchez P**, **Biernacki D**, **Lomonaco R**, **Suman A**, **Weber MH**, **Budd JT**, **Lupi ME**, **Cusi K.** Metabolic and histological implications of intrahepatic triglyceride content in nonalcoholic fatty liver disease. *Hepatology* 65: 1132–1144, 2017. doi:10.1002/hep.28985.
133. **Björntorp P.** "Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. *Arteriosclerosis* 10: 493–496, 1990. doi:10.1161/01.ATV.10.4.493.
134. **Petersen MC**, **Vatner DF**, **Shulman GI.** Regulation of hepatic glucose metabolism in health and disease. *Nat Rev Endocrinol* 13: 572–587, 2017. doi:10.1038/nrendo.2017.80.
135. **Item F**, **Konrad D** Visceral fat and metabolic inflammation: the portal theory revisited**.** *Obes Rev* 13, Suppl 2: 30–39, 2012. doi:10.1111/j.1467-789X.2012.01035.x.
136. **Kabir M**, **Catalano KJ**, **Ananthnarayan S**, **Kim SP**, **Van Citters GW**, **Dea MK**, **Bergman RN.** Molecular evidence supporting the portal theory: a causative link between visceral adiposity and hepatic insulin resistance. *Am J Physiol Endocrinol Metab* 288: E454–461, 2005. doi:10.1152/ajpendo.00203.2004.
137. **Meek SE**, **Nair KS**, **Jensen MD.** Insulin regulation of regional free fatty acid metabolism. *Diabetes* 48: 10–14, 1999. doi:10.2337/diabetes.48.1.10.
138. **Basu A**, **Basu R**, **Shah P**, **Vella A**, **Rizza RA**, **Jensen MD.** Systemic and regional free fatty acid metabolism in type 2 diabetes. *Am J Physiol Endocrinol Metab* 280: E1000–E1006, 2001. doi:10.1152/ajpendo.2001.280.6.E1000.
139. **Hodson L**, **Bickerton AS**, **McQuaid SE**, **Roberts R**, **Karpe F**, **Frayn KN**, **Fielding BA.** The contribution of splanchnic fat to VLDL triglyceride is greater in insulin-resistant than insulin-sensitive men and women: studies in the postprandial state. *Diabetes* 56: 2433–2441, 2007. doi:10.2337/db07-0654.
140. **Lytle KA**, **Bush NC**, **Triay JM**, **Kellogg TA**, **Kendrick ML**, **Swain JM**, **Gathaiya NW**, **Hames KC**, **Jensen MD.** Hepatic fatty acid balance and hepatic fat content in humans with severe obesity. *J Clin Endocrinol Metab* 104: 6171–6181, 2019. doi:10.1210/jc.2019-00875.
141. **Mardinoglu A**, **Wu H**, **Bjornson E**, **Zhang C**, **Hakkarainen A**, **Räsänen SM**, **Lee S**, **Mancina RM**, **Bergentall M**, **Pietiläinen KH**, **Söderlund S**, **Matikainen N**, **Ståhlman M**, **Bergh PO**, **Adiels M**, **Piening BD**, **Granér M**, **Lundbom N**, **Williams KJ**, **Romeo S**, **Nielsen J**, **Snyder M**, **Uhlén M**, **Bergström G**, **Perkins R**, **Marschall HU**, **Backhed F**, **Taskinen MR**, **Boren J.** An integrated understanding of the rapid metabolic benefits of a carbohydrate-restricted diet on hepatic steatosis in humans. *Cell Metab* 27: 559–571.e5, 2018. doi:10.1016/j.cmet.2018.01.005.
142. **Matikainen N**, **Adiels M**, **Söderlund S**, **Stennabb S**, **Ahola T**, **Hakkarainen A**, **Borén J**, **Taskinen MR.** Hepatic lipogenesis and a marker of hepatic lipid oxidation, predict postprandial responses of triglyceride-rich lipoproteins. *Obesity (Silver Spring)* 22: 1854–1859, 2014. doi:10.1002/oby.20781.
143. **Pramfalk C**, **Pavlides M**, **Banerjee R**, **McNeil CA**, **Neubauer S**, **Karpe F**, **Hodson L.** Fasting plasma insulin concentrations are associated with changes in hepatic fatty acid synthesis and partitioning prior to changes in liver fat content in healthy adults. *Diabetes* 65: 1858–1867, 2016. doi:10.2337/db16-0236.
144. **Pramfalk C**, **Pavlides M**, **Banerjee R**, **McNeil CA**, **Neubauer S**, **Karpe F**, **Hodson L.** Sex-specific differences in hepatic fat oxidation and synthesis may explain the higher propensity for NAFLD in men. *J Clin Endocrinol Metab* 100: 4425–4433, 2015. doi:10.1210/jc.2015-2649.
145. **Ter Horst KW**, **Gilijamse PW**, **Ackermans MT**, **Soeters MR**, **Nieuwdorp M**, **Romijn JA**, **Serlie MJ.** Impaired insulin action in the liver, but not in adipose tissue or muscle, is a distinct metabolic feature of impaired fasting glucose in obese humans. *Metab Clin Exp* 65: 757–763, 2016. doi:10.1016/j.metabol.2016.02.010.
146. **Nielsen S**, **Guo Z**, **Albu JB**, **Klein S**, **O'Brien PC**, **Jensen MD.** Energy expenditure, sex, and endogenous fuel availability in humans. *J Clin Invest* 111: 981–988, 2003. doi:10.1172/JCI16253.
147. **Fabbrini E**, **Yoshino J**, **Yoshino M**, **Magkos F**, **Tiemann Luecking C**, **Samovski D**, **Fraterrigo G**, **Okunade AL**, **Patterson BW**, **Klein S.** Metabolically normal obese people are protected from adverse effects following weight gain. *J Clin Invest* 125: 787–795, 2015. doi:10.1172/JCI78425.
148. **Gillberg L**, **Perfilyev A**, **Brons C**, **Thomasen M**, **Grunnet LG**, **Volkov P**, **Rosqvist F**, **Iggman D**, **Dahlman I**, **Riserus U**, **Ronn T**, **Nilsson E**, **Vaag A**, **Ling C.** Adipose tissue transcriptomics and epigenomics in low birthweight men and controls: role of high-fat overfeeding. *Diabetologia* 59: 799–812, 2016. doi:10.1007/s00125-015-3852-9.
149. **Johannsen DL**, **Tchoukalova Y**, **Tam CS**, **Covington JD**, **Xie W**, **Schwarz JM**, **Bajpeyi S**, **Ravussin E.** Effect of 8 weeks of overfeeding on ectopic fat deposition and insulin sensitivity: testing the "adipose tissue expandability" hypothesis. *Diabetes Care* 37: 2789–2797, 2014. doi:10.2337/dc14-0761.
150. **McLaughlin T**, **Craig C**, **Liu LF**, **Perelman D**, **Allister C**, **Spielman D**, **Cushman SW.** Adipose cell size and regional fat deposition as predictors of metabolic response to overfeeding in insulin-resistant and insulin-sensitive humans. *Diabetes* 65: 1245–1254, 2016. doi:10.2337/db15-1213.
151. **Pereira MJ**, **Skrtic S**, **Katsogiannos P**, **Abrahamsson N**, **Sidibeh CO**, **Dahgam S**, **Mansson M**, **Riserus U**, **Kullberg J**, **Eriksson JW.** Impaired adipose tissue lipid storage, but not altered lipolysis, contributes to elevated levels of NEFA in type 2 diabetes. Degree of hyperglycemia and adiposity are important factors. *Metab Clin Exp* 65: 1768–1780, 2016. doi:10.1016/j.metabol.2016.09.008.
152. **Bickerton AS**, **Roberts R**, **Fielding BA**, **Tornqvist H**, **Blaak EE**, **Wagenmakers AJ**, **Gilbert M**, **Humphreys SM**, **Karpe F**, **Frayn KN.** Adipose tissue fatty acid metabolism in insulin-resistant men. *Diabetologia* 51: 1466–1474, 2008. doi:10.1007/s00125-008-1040-x.
153. **Horowitz JF**, **Coppack SW**, **Paramore D**, **Cryer PE**, **Zhao G**, **Klein S.** Effect of short-term fasting on lipid kinetics in lean and obese women. *Am J Physiol Endocrinol Metab* 276: E278–E284, 1999. doi:10.1152/ajpendo.1999.276.2.E278.
154. **Jocken JW**, **Goossens GH**, **van Hees AM**, **Frayn KN**, **van BM**, **Stegen J**, **Pakbiers MT**, **Saris WH**, **Blaak EE.** Effect of beta-adrenergic stimulation on whole-body and abdominal subcutaneous adipose tissue lipolysis in lean and obese men. *Diabetologia* 51: 320–327, 2008. doi:10.1007/s00125-007-0866-y.
155. **Camell CD**, **Sander J**, **Spadaro O**, **Lee A**, **Nguyen KY**, **Wing A**, **Goldberg EL**, **Youm YH**, **Brown CW**, **Elsworth J**, **Rodeheffer MS**, **Schultze JL**, **Dixit VD.** Inflammasome-driven catecholamine catabolism in macrophages blunts lipolysis during ageing. *Nature* 550: 119–123, 2017. doi:10.1038/nature24022.
156. **Gastaldelli A**, **Casolaro A**, **Ciociaro D**, **Frascerra S**, **Nannipieri M**, **Buzzigoli E**, **Ferrannini E.** Decreased whole body lipolysis as a mechanism of the lipid-lowering effect of pioglitazone in type 2 diabetic patients. *Am J Physiol Endocrinol Metab* 297: E225–E230, 2009. doi:10.1152/ajpendo.90960.2008.
157. **Brassard P**, **Frisch F**, **Lavoie F**, **Cyr D**, **Bourbonnais A**, **Cunnane SC**, **Patterson BW**, **Drouin R**, **Baillargeon JP**, **Carpentier AC.** Impaired plasma nonesterified fatty acid tolerance is an early defect in the

natural history of type 2 diabetes. *J Clin Endocrinol Metab* 93: 837–844, 2008. doi:10.1210/jc.2007-1670.

158. **Gepner Y**, **Shelef I**, **Schwarzfuchs D**, **Zelicha H**, **Tene L**, **Yaskolka Meir A**, **Tsaban G**, **Cohen N**, **Bril N**, **Rein M**, **Serfaty D**, **Kenigsbuch S**, **Komy O**, **Wolak A**, **Chassidim Y**, **Golan R**, **Avni-Hassid H**, **Bilitzky A**, **Sarusi B**, **Goshen E**, **Shemesh E**, **Henkin Y**, **Stumvoll M**, **Bluher M**, **Thiery J**, **Ceglarek U**, **Rudich A**, **Stampfer MJ**, **Shai I.** Effect of distinct lifestyle interventions on mobilization of fat storage pools: CENTRAL Magnetic Resonance Imaging Randomized Controlled Trial. *Circulation* 137: 1143–1157, 2018. doi:10.1161/CIRCULATIONAHA.117.030501.
159. **Coppack SW**, **Evans RD**, **Fisher RM**, **Frayn KN**, **Gibbons GF**, **Humphreys SM**, **Kirk ML**, **Potts JL**, **Hockaday TD.** Adipose tissue metabolism in obesity: lipase action in vivo before and after a mixed meal. *Metab Clin Exp* 41: 264–272, 1992. doi:10.1016/0026-0495(92)90269-G.
160. **Frayn KN**, **Humphreys SM**, **Coppack SW.** Net carbon flux across subcutaneous adipose tissue after a standard meal in normal-weight and insulin-resistant obese subjects. *Int J Obes Relat Metab Disord* 20: 795–800, 1996.
161. **McQuaid SE**, **Hodson L**, **Neville MJ**, **Dennis AL**, **Cheeseman J**, **Humphreys SM**, **Ruge T**, **Gilbert M**, **Fielding BA**, **Frayn KN**, **Karpe F.** Downregulation of adipose tissue fatty acid trafficking in obesity: a driver for ectopic fat deposition? *Diabetes* 60: 47–55, 2011. doi:10.2337/db10-0867.
162. **Coppack SW**, **Fisher RM**, **Humphreys SM**, **Clark ML**, **Pointon JJ**, **Frayn KN.** Carbohydrate metabolism in insulin resistance: glucose uptake and lactate production by adipose and forearm tissues in vivo before and after a mixed meal. *Clin Sci (Lond)* 90: 409–415, 1996. doi:10.1042/cs0900409.
163. **Karpe F**, **Fielding BA**, **Ilic V**, **Macdonald IA**, **Summers LK**, **Frayn KN.** Impaired postprandial adipose tissue blood flow response is related to aspects of insulin sensitivity. *Diabetes* 51: 2467–2473, 2002. doi:10.2337/diabetes.51.8.2467.
164. **Ardilouze JL**, **Sotornik R**, **Dennis LA**, **Fielding BA**, **Frayn KN**, **Karpe F.** Failure to increase postprandial blood flow in subcutaneous adipose tissue is associated with tissue resistance to adrenergic stimulation. *Diabetes Metab* 38: 27–33, 2012. doi:10.1016/j.diabet.2011.06.005.
165. **Funada J**, **Dennis AL**, **Roberts R**, **Karpe F**, **Frayn KN.** Regulation of subcutaneous adipose tissue blood flow is related to measures of vascular and autonomic function. *Clin Sci (Lond)* 119: 313–322, 2010. doi:10.1042/CS20100066.
166. **Søndergaard E**, **Johansen RF**, **Jensen MD**, **Nielsen S.** Postprandial VLDL-TG metabolism in type 2 diabetes. *Metabolism* 75: 25–35, 2017. doi:10.1016/j.metabol.2017.07.002.
167. **Tuvdendorj D**, **Chandalia M**, **Batbayar T**, **Saraf M**, **Beysen C**, **Murphy EJ**, **Abate N.** Altered subcutaneous abdominal adipose tissue lipid synthesis in obese, insulin-resistant humans. *Am J Physiol Endocrinol Metab* 305: E999–E1006, 2013. doi:10.1152/ajpendo.00194.2013.
168. **Allister CA**, **Liu LF**, **Lamendola CA**, **Craig CM**, **Cushman SW**, **Hellerstein MK**, **McLaughlin TL.** In vivo $^{2}H_{2}O$ administration reveals impaired triglyceride storage in adipose tissue of insulin-resistant humans. *J Lipid Res* 56: 435–439, 2015. doi:10.1194/jlr.M052860.
169. **Nouws J**, **Fitch M**, **Mata M**, **Santoro N**, **Galuppo B**, **Kursawe R**, **Narayan D**, **Vash-Margita A**, **Pierpont B**, **Shulman GI**, **Hellerstein M**, **Caprio S.** Altered in vivo lipid fluxes and cell dynamics in subcutaneous adipose tissues are associated with the unfavorable pattern of fat distribution in obese adolescent girls. *Diabetes* 68: 1168–1177, 2019. doi:10.2337/db18-1162.
170. **Labbé SM**, **Grenier-Larouche T**, **Noll C**, **Phoenix S**, **Guérin B**, **Turcotte EE**, **Carpentier AC.** Increased myocardial uptake of dietary fatty acids linked to cardiac dysfunction in glucose-intolerant humans. *Diabetes* 61: 2701–2710, 2012. doi:10.2337/db11-1805.
171. **Noll C**, **Carpentier AC.** Dietary fatty acid metabolism in prediabetes. *Curr Opin Lipidol* 28: 1–10, 2017. doi:10.1097/MOL.0000000000000369.
172. **Labbé SM**, **Noll C**, **Grenier-Larouche T**, **Kunach M**, **Bouffard L**, **Phoenix S**, **Guerin B**, **Baillargeon JP**, **Langlois MF**, **Turcotte EE**, **Carpentier AC.** Improved cardiac function and dietary fatty acid metabolism after modest weight loss in subjects with impaired glucose tolerance. *Am J Physiol Endocrinol Metab* 306: E1388–E1396, 2014. doi:10.1152/ajpendo.00638.2013.
173. **Miedema MD**, **Maziarz M**, **Biggs ML**, **Zieman SJ**, **Kizer JR**, **Ix JH**, **Mozaffarian D**, **Tracy RP**, **Psaty BM**, **Siscovick DS**, **Mukamal KJ**, **Djousse L.** Plasma-free fatty acids, fatty acid-binding protein 4, and mortality in older adults (from the Cardiovascular Health Study). *Am J Cardiol* 114: 843–848, 2014. doi:10.1016/j.amjcard.2014.06.012.
174. **Deelen J**, **Kettunen J**, **Fischer K**, **van der Spek A**, **Trompet S**, **Kastenmuller G**, et al**.** A metabolic profile of all-cause mortality risk identified in an observational study of 44,168 individuals. *Nat Commun* 10: 3346, 2019. doi:10.1038/s41467-019-11311-9.
175. **Xiao C**, **Giacca A**, **Carpentier A**, **Lewis GF.** Differential effects of monounsaturated, polyunsaturated and saturated fat ingestion on glucose-stimulated insulin secretion, sensitivity and clearance in overweight and obese, non-diabetic humans. *Diabetologia* 49: 1371–1379, 2006. doi:10.1007/s00125-006-0211-x.
176. **Halliwell KJ**, **Fielding BA**, **Samra JS**, **Humphreys SM**, **Frayn K.** Release of individual fatty acids from human adipose tissue in vivo after an overnight fast. *J Lipid Res* 37: 1842–1848, 1996. doi:10.1016/S0022-2275(20)37549-0.
177. **Hodson L**, **McQuaid SE**, **Karpe F**, **Frayn KN**, **Fielding BA.** Differences in partitioning of meal fatty acids into blood lipid fractions: a comparison of linoleate, oleate, and palmitate. *Am J Physiol Endocrinol Metab* 296: E64–E71, 2009. doi:10.1152/ajpendo.90730.2008.
178. **Murashige D**, **Jang C**, **Neinast M**, **Edwards JJ**, **Cowan A**, **Hyman MC**, **Rabinowitz JD**, **Frankel DS**, **Arany Z.** Comprehensive quantification of fuel use by the failing and nonfailing human heart. *Science* 370: 364–368, 2020. doi:10.1126/science.abc8861.
179. **Walker CG**, **Browning LM**, **Stecher L**, **West AL**, **Madden J**, **Jebb SA**, **Calder PC.** Fatty acid profile of plasma NEFA does not reflect adipose tissue fatty acid profile. *Br J Nutr* 114: 756–762, 2015. doi:10.1017/S0007114515002251.
180. **Chong MF**, **Hodson L**, **Bickerton AS**, **Roberts R**, **Neville M**, **Karpe F**, **Frayn KN**, **Fielding BA.** Parallel activation of de novo lipogenesis and stearoyl-CoA desaturase activity after 3 d of high-carbohydrate feeding. *Am J Clin Nutr* 87: 817–823, 2008. doi:10.1093/ajcn/87.4.817.
181. **Collins JM**, **Neville MJ**, **Hoppa MB**, **Frayn KN.** De novo lipogenesis and stearoyl-CoA desaturase are coordinately regulated in the human adipocyte and protect against palmitate-induced cell injury. *J Biol Chem* 285: 6044–6052, 2010. doi:10.1074/jbc.M109.053280.
182. **Bjermo H**, **Iggman D**, **Kullberg J**, **Dahlman I**, **Johansson L**, **Persson L**, **Berglund J**, **Pulkki K**, **Basu S**, **Uusitupa M**, **Rudling M**, **Arner P**, **Cederholm T**, **Ahlstrom H**, **Riserus U.** Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial. *Am J Clin Nutr* 95: 1003–1012, 2012. doi:10.3945/ajcn.111.030114.
183. **Iggman D**, **Rosqvist F**, **Larsson A**, **Arnlov J**, **Beckman L**, **Rudling M**, **Riserus U.** Role of dietary fats in modulating cardiometabolic risk during moderate weight gain: a randomized double-blind overfeeding trial (LIPOGAIN study). *J Am Heart Assoc* 3: e001095, 2014.
184. **Luukkonen PK**, **Sädevirta S**, **Zhou Y**, **Kayser B**, **Ali A**, **Ahonen L**, **Lallukka S**, **Pelloux V**, **Gaggini M**, **Jian C**, **Hakkarainen A**, **Lundbom N**, **Gylling H**, **Salonen A**, **Orešič M**, **Hyötyläinen T**, **Orho-Melander M**, **Rissanen A**, **Gastaldelli A**, **Clement K**, **Hodson L**, **Yki-Järvinen H.** Saturated fat is more metabolically harmful for the human liver than unsaturated fat or simple sugars.*Diabetes Care* 41: 1732–1739, 2018. doi:10.2337/dc18-0071.
185. **Rosqvist F**, **Iggman D**, **Kullberg J**, **Cedernaes J**, **Johansson HE**, **Larsson A**, **Johansson L**, **Ahlstrom H**, **Arner P**, **Dahlman I**, **Risérus U.** Overfeeding polyunsaturated and saturated fat causes distinct effects on liver and visceral fat accumulation in humans. *Diabetes* 63: 2356–2368, 2014. doi:10.2337/db13-1622.
186. **Rosqvist F**, **Kullberg J**, **Ståhlman M**, **Cedernaes J**, **Heurling K**, **Johansson HE**, **Iggman D**, **Wilking H**, **Larsson A**, **Eriksson O**, **Johansson L**, **Straniero S**, **Rudling M**, **Antoni G**, **Lubberink M**, **Orho-Melander M**, **Boren J**, **Ahlström H**, **Riserus U.** Overeating saturated fat promotes fatty liver and ceramides compared with polyunsaturated fat: a randomized trial. *J Clin Endocrinol Metab* 104: 6207–6219, 2019. doi:10.1210/jc.2019-00160.
187. **Hellerstein MK**, **Schwarz JM**, **Neese RA.** Regulation of hepatic de novo lipogenesis in humans. *Annu Rev Nutr* 16: 523–557, 1996. doi:10.1146/annurev.nu.16.070196.002515.
188. **Hudgins LC**, **Hellerstein MK**, **Seidman CE**, **Neese RA**, **Tremaroli JD**, **Hirsch , J.** Relationship between carbohydrate-induced hypertriglyce-

ridemia and fatty acid synthesis in lean and obese subjects. *J Lipid Res* 41: 595–604, 2000. doi:10.1016/S0022-2275(20)32407-X.

189. **Stanhope KL**, **Schwarz JM**, **Keim NL**, **Griffen SC**, **Bremer AA**, **Graham JL**, **Hatcher B**, **Cox CL**, **Dyachenko A**, **Zhang W**, **McGahan JP**, **Seibert A**, **Krauss RM**, **Chiu S**, **Schaefer EJ**, **Ai M**, **Otokozawa S**, **Nakajima K**, **Nakano T**, **Beysen C**, **Hellerstein MK**, **Berglund L**, **Havel PJ.** Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest* 119: 1322–1334, 2009. doi:10.1172/JCI37385.
190. **Yki-Jarvinen H.** Nutritional modulation of non-alcoholic fatty liver disease and insulin resistance. *Nutrients* 7: 9127–9138, 2015. doi:10.3390/nu7115454.
191. **Seidelmann SB**, **Claggett B**, **Cheng S**, **Henglin M**, **Shah A**, **Steffen LM**, **Folsom AR**, **Rimm EB**, **Willett WC**, **Solomon SD.** Dietary carbohydrate intake and mortality: a prospective cohort study and meta-analysis. *Lancet Public Health* 3: e419–e428, 2018. doi:10.1016/S2468-2667(18)30135-X.
192. **Viana-Huete V**, **Guillén C**, **Garcia G**, **Fernández S**, **García-Aguilar A**, **Kahn CR**, **Benito M.** Male brown fat-specific double knockout of IGFIR/IR: atrophy, mitochondrial fission failure, impaired thermogenesis, and obesity. *Endocrinology* 159: 323–340, 2018. doi:10.1210/en.2017-00738.
193. **Carpentier AC**, **Blondin DP**, **Virtanen KA**, **Richard D**, **Haman F**, **Turcotte EE.** Brown adipose tissue energy metabolism in humans. *Front Endocrinol (Lausanne)* 9: 447, 2018. doi:10.3389/fendo.2018.00447.
194. **Blondin DP**, **Frisch F**, **Phoenix S**, **Guérin B**, **Turcotte EE**, **Haman F**, **Richard D**, **Carpentier AC.** Inhibition of intracellular triglyceride lipolysis suppresses cold-induced brown adipose tissue metabolism and increases shivering in humans. *Cell Metab* 25: 438–447, 2017. doi:10.1016/j.cmet.2016.12.005.
195. **Labbé SM**, **Caron A**, **Bakan I**, **Laplante M**, **Carpentier AC**, **Lecomte R**, **Richard D.** In vivo measurement of energy substrate contribution to cold-induced brown adipose tissue thermogenesis. *FASEB J* 29: 2046–2058, 2015. doi:10.1096/fj.14-266247.
196. **Dallon BW**, **Parker BA**, **Hodson AE**, **Tippetts TS**, **Harrison ME**, **Appiah MMA**, **Witt JE**, **Gibbs JL**, **Gray HM**, **Sant TM**, **Bikman BT.** Insulin selectively reduces mitochondrial uncoupling in brown adipose tissue in mice. *Biochem J* 475: 561–569, 2018. doi:10.1042/BCJ20170736.
197. **Orava J**, **Nuutila P**, **Lidell ME**, **Oikonen V**, **Noponen T**, **Viljanen T**, **Scheinin M**, **Taittonen M**, **Niemi T**, **Enerback S**, **Virtanen KA.** Different metabolic responses of human brown adipose tissue to activation by cold and insulin. *Cell Metab* 14: 272–279, 2011. doi:10.1016/j.cmet.2011.06.012.
198. **Orava J**, **Nuutila P**, **Noponen T**, **Parkkola R**, **Viljanen T**, **Enerbäck S**, **Rissanen A**, **Pietilainen KH**, **Virtanen KA.** Blunted metabolic responses to cold and insulin stimulation in brown adipose tissue of obese humans. *Obesity (Silver Spring)* 21: 2279–2287, 2013. doi:10.1002/oby.20456.
199. **U. Din M**, **Saari T**, **Raiko J**, **Kudomi N**, **Maurer SF**, **Lahesmaa M**, **Fromme T**, **Amri EZ**, **Klingenspor M**, **Solin O**, **Nuutila P**, **Virtanen KA.** Postprandial oxidative metabolism of human brown fat indicates thermogenesis. *Cell Metab* 28: 207–216.e3, 2018. doi:10.1016/j.cmet.2018.05.020.
200. **Heine M**, **Fischer AW**, **Schlein C**, **Jung C**, **Straub LG**, **Gottschling K**, **Mangels N**, **Yuan Y**, **Nilsson SK**, **Liebscher G**, **Chen O**, **Schreiber R**, **Zechner R**, **Scheja L**, **Heeren J.** Lipolysis triggers a systemic insulin response essential for efficient energy replenishment of activated brown adipose tissue in mice. *Cell Metab* 28: 644–655 e644, 2018. doi:10.1016/j.cmet.2018.06.020.
201. **Blondin DP**, **Tingelstad HC**, **Noll C**, **Frisch F**, **Phoenix S**, **Guérin B**, **Turcotte EE**, **Richard D**, **Haman F**, **Carpentier AC.** Dietary fatty acid metabolism of brown adipose tissue in cold-acclimated men. *Nat Commun* 8: 14146, 2017. doi:10.1038/ncomms14146.
202. **Ouellet V**, **Labbé SM**, **Blondin DP**, **Phoenix S**, **Guérin B**, **Haman F**, **Turcotte EE**, **Richard D**, **Carpentier AC.** Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. *J Clin Invest* 122: 545–552, 2012. doi:10.1172/JCI60433.
203. **Gamble J**, **Lopaschuk GD.** Insulin inhibition of 5' adenosine monophosphate-activated protein kinase in the heart results in activation of acetyl coenzyme A carboxylase and inhibition of fatty acid oxidation. *Metab Clin Exp* 46: 1270–1274, 1997. doi:10.1016/S0026-0495(97)90229-8.
204. **Sidossis LS**, **Mittendorfer B**, **Chinkes D**, **Walser E**, **Wolfe RR.** Effect of hyperglycemia-hyperinsulinemia on whole body and regional fatty acid metabolism. *Am J Physiol Endocrinol Metab* 276: E427–E434, 1999. doi:10.1152/ajpendo.1999.276.3.E427.
205. **Sidossis LS**, **Stuart CA**, **Shulman GI**, **Lopaschuk GD**, **Wolfe R.** Glucose plus insulin regulate fat oxidation by controlling the rate of fatty acid entry into the mitochondria. *J Clin Invest* 98: 2244–2250, 1996. doi:10.1172/JCI119034.
206. **Sidossis LS**, **Wolfe RR.** Glucose and insulin-induced inhibition of fatty acid oxidation: the glucose-fatty acid cycle reversed. *Am J Physiol Endocrinol Metab* 270: E733–E738, 1996. doi:10.1152/ajpendo.1996.270.4.E733.
207. **Blondin DP**, **Nielsen S**, **Kuipers EN**, **Severinsen MC**, **Jensen VH**, **Miard S**, **Jespersen NZ**, **Kooijman S**, **Boon MR**, **Fortin M**, **Phoenix S**, **Frisch F**, **Guerin B**, **Turcotte EE**, **Haman F**, **Richard D**, **Picard F**, **Rensen PCN**, **Scheele C**, **Carpentier AC.** Human brown adipocyte thermogenesis is driven by β2-AR stimulation. *Cell Metab* 32: 287–300 e287, 2020. doi:10.1016/j.cmet.2020.07.005.