subcutaneous fat, but participants with diabetes had significantly more abdominal visceral fat (2.9 kg vs 2.4 kg; p<0.001) and liver fat (8.3% vs 4.8%; p<0.001) than those without diabetes (Table 1) [Neeland IJ et al. *JAMA* 2012]. Among individuals without diabetes at baseline, those with the highest amount of visceral fat were at greatest risk for developing diabetes (p<0.001).

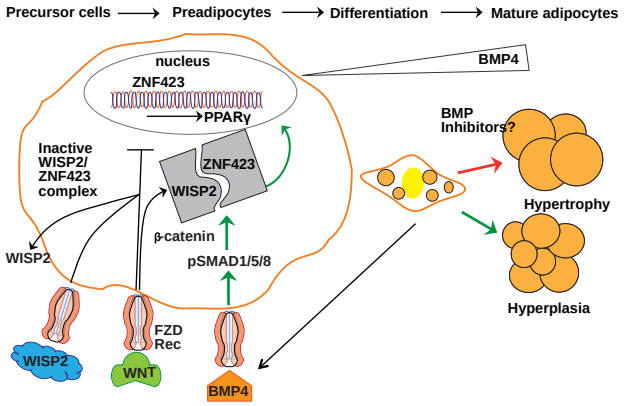
Table 1. Baseline Characteristics of Obese Patients With and Without Incident Type 2 Diabetes

	No Diabetes (n=648)	Incident Diabetes (n=84)	p Value
DEXA fat measures, median (IQR)			
Total fat mass, kg	35.5 (29.3–43.4)	35.3 (28.8–42.7)	0.51
Total lean mass, kg	57.3 (50.0–67.6)	58.84 (52.7–70.2)	0.10
Body fat, %	40.4 (31.6–44.5)	39.8 (28.7–43.8)	0.51
Lower body fat mass, kg	12.6 (9.6–16.3)	11.2 (9.0–15.1)	0.02
Truncal fat mass, kg	17.4 (14.8–21.4)	17.9 (15.8–21.9)	0.54
MRI fat measures, median (IQR)			
Abdominal subcutaneous fat, kg	6.5 (5.0–8.8)	6.9 (4.8–8.9)	0.88
Abdominal visceral fat, kg	2.4 (1.9–3.1)	2.9 (2.5–3.4)	<0.001
Liver fat, %	4.8 (3.1–8.7)	8.3 (4.6–14.4)	<0.001
Cardiac and vascular MRI measures, median (IQR)			
LV mass/BSA, g/m^2	76.6 (68.3–87.3)	82.2 (74.2–93.1)	0.003
LV wall thickness, mm	11.6 (10.7–12.8)	12.4 (11.2–13.6)	<0.001
Aortic compliance, mL/mm Hg	24.4 (17.2–32.7)	19.7 (15.1–28.2)	0.01

A study comparing lean and overweight individuals found that those with a genetic predisposition for type 2 diabetes had restricted adipogenesis and hypertrophic obesity even in the absence of obesity as defined by body mass index (BMI) [Arner P et al. *PloS One* 2011].

Restricted adipogenesis is not due to a lack of precursor cells but to an inability to recruit and differentiate subcutaneous preadipocytes [Isakson P et al. *Diabetes* 2009]. This inability is caused by inadequate signaling and activation of bone morphogenetic protein 4 (BMP4) and inadequate suppression of canonical Wnt signaling [Gustafson B et al. *Diabetes* 2013]. BMP4 is produced by preadipocytes and adipocytes, and it induces precursor cell commitment to the adipocyte lineage. Canonical Wnt prevents activation and differentiation of preadipocytes by peroxisome proliferator-activated receptor gamma (PPAR-γ). The Wnt-inducible secreted protein 2 (WISP2), expressed in preadipocytes, inhibits adipogenesis by forming a complex with the transcriptional activator of PPAR-γ, ZNF423. BMP4 dissociates this complex, resulting in entry of ZNF423 into the nucleus where it initiates PPAR-γ activation. WISP2 also directly inhibits PPAR-γ activation (Figure 1) [Gustafson B et al. *Diabetes* 2013].

Figure 1. Recruitment and Differentiation of Preadipocytes



Adapted from Gustafson B et al. *Diabetes* 2013.

These studies shed new light on the mechanisms of ectopic fat accumulation and development of hypertrophic obesity. Hypertrophic obesity is associated with dysregulated adipose tissue with reduced local and systemic insulin sensitivity regardless of the amount of body fat. The ability to recruit new subcutaneous adipocytes protects against ectopic fat accumulation, inflammation, and insulin resistance.

Oversupply of Free Fatty Acids to the Systemic Circulation Is Not a Distinct Feature of Insulin Resistance or Obesity

Written by Toni Rizzo

Measurements of free fatty acid (FFA), also known to as non-esterified fatty acid, and glucose kinetics have shown that obese individuals have adipose tissue that is insulin resistant [Jensen MD, Nielsen S. *Metabolism* 2007]. It is commonly thought that oversupply of FFA is a major contributor to the development of insulin resistance in obesity. Once insulin resistance develops, lipolysis of stored triacylglycerol is increased, resulting in increased release of FFAs by adipocytes [Eckel RH et al. *Lancet* 2005]. Fredrik Karpe, MD, PhD, Oxford University, Oxford, United Kingdom, discussed the known molecular mechanisms for the phenomenon of insulin resistance in adipose tissue and challenged the notion of oversupply of FFA in obesity.

A study comparing lean versus abdominally obese men from the Oxford Biobank found a clear difference in fat mass and insulin resistance but no difference in

FFA concentrations between the 2 groups [McQuaid S et al. *Diabetes* 2011]. A systematic literature review and comparison of patients from the Oxford Biobank found no relationship between body mass index (BMI) and FFA concentrations [Karpe F et al. *Diabetes* 2011]. Additionally, an analysis of several large studies found that hyperinsulinemia was associated with a decreased release of FFA from adipose tissue [Karpe F et al. *Diabetes* 2011]. In fact, adipose tissue in obese (and insulin resistant) individuals almost invariably supply less FFA per unit fat mass than in lean individuals.

Prof. Karpe and fellow researchers investigated whether excess fat deposition in non-adipose tissue is due to excess fatty acid delivery from adipose tissue or to impaired adipose tissue fat storage [McQuaid S et al. *Diabetes* 2011]. Using stable-isotope fatty acid tracers to assess FFA delivery over a diurnal cycle, they found that the FFA Ra (rate of appearance) was significantly higher in abdominally obese versus lean men ($p=0.009$), but when the data were normalized per lean body mass, the difference disappeared. When expressed per total fat mass, the obese men had significantly lower FFA Ra compared with lean men ($p=0.029$). It therefore appears that adipose tissue in obese individuals down-regulate FFA supply to the systemic circulation as part of an adequate metabolic adaptation despite an element of insulin resistance.

In the same study, lean men had a progressive increase in meal fat deposition into adipose tissue with each meal (13%, 35%, and 47%, first to third meal; $p<0.001$). Abdominally obese men did not have a significant increase in adipose tissue fat storage with sequential feeding (6%, 25%, and 18%, first to third meal; $p=0.12$). The difference was statistically significant for the last meal ($p=0.001$). As the up-regulation of fat storage is an insulin-sensitive process, it therefore seems that obese individuals have a defect in immediate fat storage. The transcriptional signature of adipose tissue from the obese men was consistent with impaired fat storage function. Klimcakova and colleagues demonstrated that lipolysis, lipogenesis, glycolysis, and mitochondrial genes are highly downregulated in obese adipose tissue [Klimcakova E et al. *J Clin Endocrinol Metab* 2011].

According to Prof. Karpe, analysis of available evidence shows that adipose tissues and organs do not oversecrete FFA but instead adapt extremely well to obesity. Adipose tissue can be insulin resistant but in terms of regulation of lipolysis, this is balanced by hyperinsulinemia. Human adipose tissue adapts to obesity, hyperplasia, and hyperinsulinemia by down-regulating metabolic processes. Increased release of FFA is not an obvious feature in the development of insulin resistance, while adipose tissue fat storage is decreased in obese individuals leading to ectopic fat deposition, with the liver as the prime target.

PPAR-Mediated Mechanisms of Skeletal Muscle Insulin Resistance

Written by **Nicola Parry**

Skeletal muscle is the major site for insulin-dependent glucose utilization, and insulin resistance in skeletal muscle is thought to be integral in the pathogenesis of type 2 diabetes mellitus (T2DM). Kyong Soo Park, MD, PhD, Seoul National University, Seoul, South Korea, discussed some emerging insights into the molecular basis of skeletal muscle insulin resistance, highlighting the role of peroxisome proliferator activated receptor–gamma (PPAR-γ), a nuclear transcription factor.

PPAR-γ is implicated in the regulation of fat metabolism in skeletal muscle, and is activated by the binding of specific ligands, including thiazolidinediones (TZDs), such as troglitazone, rosiglitazone, and pioglitazone, which are used for the treatment of T2DM [Chung SS et al. *Mol Cell Biol* 2009]. Although PPAR-γ is mostly expressed in fat tissue where it is critically involved in lipogenesis and adipocyte differentiation, low levels are also expressed in skeletal muscle where it enhances insulin sensitivity [Chung SS et al. *Biochem J* 2011].

Oxidative stress plays a central role in the pathogenesis of insulin resistance, T2DM, and its vascular complications. Production of reactive oxygen species is increased in T2DM, and antioxidant activity is simultaneously reduced. TZDs, however, have been shown to prevent oxidative stress-induced insulin resistance in skeletal muscle. They activate PPAR-γ, stimulating glutathione peroxidase 3 (GPx3) gene expression leading to reduction of extracellular hydrogen peroxide (H_2O_2) levels that contribute to insulin resistance in skeletal muscle cells. Since inhibition of GPx3 expression prevents this TZD-induced antioxidant effect, GPx3 is thought to be essential for the regulation of PPAR-γ-mediated antioxidant activity. And since lower GPx3 levels have also been demonstrated in patients with T2DM and in diabetic diet-induced obese mice, the antioxidant effect of PPAR-γ is considered to be completely mediated by GPx3 [Chung SS et al. *Mol Cell Biol* 2009].

More recently, the function of PPAR-γ has been shown to be regulated by various posttranslational modifications, including SUMOylation, which involves binding of a small ubiquitin-like modifier (SUMO) protein to target proteins. SUMOylation is catalyzed by SUMO-specific proteases (SENPs). In one study of C2C12 cells in a primary line of mouse myoblasts, the critical role of SENP2 in lipogenesis as a desumoylating enzyme was demonstrated. SENP2 effectively removed SUMO from PPAR-γ–SUMO conjugates, while also increasing PPAR-γ transcriptional activity. In