

# In Muscle-Specific Lipoprotein Lipase—Overexpressing Mice, Muscle Triglyceride Content Is Increased Without Inhibition of Insulin-Stimulated Whole-Body and Muscle-Specific Glucose Uptake

Peter J. Voshol,<sup>1,2</sup> Miek C. Jong,<sup>1</sup> Vivian E.H. Dahlmans,<sup>1</sup> Dagmar Kratky,<sup>3</sup> Sanja Levak-Frank,<sup>4</sup> Rudolf Zechner,<sup>3</sup> Johannes A. Romijn,<sup>2</sup> and Louis M. Havekes<sup>1,2</sup>

In patients with type 2 diabetes, a strong correlation between accumulation of intramuscular triglycerides (TGs) and insulin resistance has been found. The aim of the present study was to determine whether there is a causal relation between intramuscular TG accumulation and insulin sensitivity. Therefore, in mice with muscle-specific overexpression of human lipoprotein lipase (LPL) and control mice, muscle TG content was measured in combination with glucose uptake *in vivo*, under hyperinsulinemic-euglycemic conditions. Overexpression of LPL in muscle resulted in accumulation of TGs in skeletal muscle ( $85.5 \pm 33.3$  vs.  $25.7 \pm 23.1$   $\mu\text{mol/g}$  tissue in LPL and control mice, respectively;  $P < 0.05$ ). During the hyperinsulinemic clamp study, there were no differences in plasma glucose, insulin, and FFA concentrations between the two groups. Moreover, whole-body, as well as skeletal muscle, insulin-mediated glucose uptake did not differ between LPL-overexpressing and wild-type mice. Surprisingly, whole-body glucose oxidation was decreased by  $\sim 60\%$  ( $P < 0.05$ ), whereas nonoxidative glucose disposal was increased by  $\sim 50\%$  ( $P < 0.05$ ) in LPL-overexpressing versus control mice. In conclusion, overexpression of human LPL in muscle increases intramuscular TG accumulation, but does not affect whole-body or muscle-specific insulin-mediated uptake, findings that argue against a simple causal relation between intramuscular TG content and insulin resistance. *Diabetes* 50:2585–2590, 2001

Altered partitioning of triglycerides (TGs) between adipose tissue and nonadipose tissues, like muscle or liver, is hypothesized to be involved in the development of insulin resistance associated with obesity and type 2 diabetes (1–3). In patients with type 2 diabetes, there is a strong correlation between accumulation of intramuscular TGs and insulin resistance (4,5). A causal relation between muscle TG content and insulin resistance is supported by observations in mice (6) and rats (7). In lipotrophic mice, hyperglycemia and hyperinsulinemia develop in association with excessive accumulation of TGs in muscle and liver (6). Reversal of this muscle TG accumulation by adipose tissue transplantation reverses the biochemical features of type 2 diabetes in these mice (8). Finally, these changes in TG content in lipotrophic mice induce opposite changes in phosphatidylinositol 3-kinase activity in muscle, indicating a possible causal relationship between muscle TG content and insulin resistance (9). Rats fed a diet high in saturated fats and/or subjected to prolonged inhibition of fatty acid oxidation have also shown a correlation between insulin action and intramuscular TG content (7). Therefore, TG accumulation in tissues other than adipose tissue might provide a causal link in the association between obesity and diabetes (10,11).

Lipoprotein lipase (LPL) is most abundant in adipose tissue and muscle (12,13). LPL mediates lipolysis of TGs from TG-rich lipoproteins, resulting in the release of fatty acids from these lipoproteins (12,13). In this way, LPL controls entry of fatty acids into underlying tissues (adipose, muscle) (1,14), where the fatty acids can be stored in TGs (adipose tissue) or oxidized (muscle tissue) (1,15). It has been hypothesized that the relative levels of LPL within adipose and muscle tissue determine the partitioning of plasma TGs between these tissues (14,16,17). Consequently, modulation of muscle-specific LPL expression is a unique tool for manipulating muscle fatty acid uptake and, consequently, muscle TG content.

The aim of the present study was to evaluate the causality of the relation between muscle TG content and insulin sensitivity in a nonlipotrophic mouse model. Therefore, we studied mice that overexpress LPL specifically in muscle (18,19) as a model for altered partitioning

From <sup>1</sup>TNO-Prevention and Health, Division VBO, Leiden, the Netherlands; the <sup>2</sup>Department of Endocrinology and Metabolic Diseases, Leiden University Medical Centre, Leiden, the Netherlands; <sup>3</sup>Institute of Molecular Biology, Biochemistry, and Microbiology, University of Graz, Graz, Austria; and <sup>4</sup>Institute of Medical Biochemistry and Medical Molecular Biology, University of Graz, Graz, Austria.

Address correspondence and reprint requests to Dr. Peter J. Voshol, TNO-Prevention and Health, Division VBO, Zernikedeerf 9, NL-2333 CK Leiden, The Netherlands. E-mail: pj.voshol@pg.tno.nl.

Received for publication 14 March 2001 and accepted in revised form 10 August 2001.

AGAT, acylglycerol-3-phosphate acyltransferase; 2-DG, 2-deoxy-D-[1-<sup>14</sup>C]glucose; 2-DG-P, 2-DG-6-phosphate; EGP, endogenous glucose production; FFA, free fatty acid; LPL, lipoprotein lipase; PBS, phosphate-buffered saline; TG, triglyceride.

TABLE 1

Plasma total cholesterol, TG, FFA, glucose, and insulin concentrations measured in overnight-fasted muscle-specific LPL-overexpressing and control (wild-type) mice

Mouse	<i>n</i>	Body weight (g)	Total cholesterol (mmol/l)	TG (mmol/l)	FFA (mmol/l)	Glucose (mmol/l)	Insulin (pmol/l)
Wild-type	8	30.6 ± 2.1	2.6 ± 0.6	0.70 ± 0.3	1.1 ± 0.2	5.7 ± 1.3	53 ± 15
LPL-TG	6	28.6 ± 2.7	1.4 ± 0.4*	0.12 ± 0.13*	0.9 ± 0.2	6.1 ± 0.9	97 ± 11*

Data are means ± SD. LPL-TG, LPL-overexpressing transgenic mice. \**P* < 0.05 vs. wild-type, as assessed by the Mann-Whitney *U* test.

of TGs toward muscle tissue (20,21). We found that in these mice, muscle TG content was indeed significantly increased. However, the basal and insulin-mediated glucose uptake in these mice was not affected as compared with control mice, indicating the absence of a simple causal relationship between muscle TG stores and insulin sensitivity.

## RESEARCH DESIGN AND METHODS

**Animals.** Male, muscle-specific human LPL-overexpressing C6/CBA mice on an endogenous murine LPL (LPL-TG or L2-MCK) background (18) and wild-type mice, ages 4–6 months, were taken from the breeding colony at the University of Graz, Austria. Mice were kept in a temperature- and humidity-controlled environment and had free access to standard laboratory diet and water. All animal experiments were approved by the Animal Ethics Committee of TNO-Prevention and Health, Leiden, the Netherlands.

**Fasted plasma parameters.** After mice were fasted overnight, 150- $\mu$ l blood samples were taken in paraoxon-coated capillaries via tail bleeding from the different genotypes. Plasma was collected by centrifugation, and total plasma glucose, free fatty acids (FFAs), TGs, and total cholesterol were determined via commercially available kits (Sigma, St. Louis, MO; Boehringer Mannheim, Mannheim, Germany; and Wako Chemicals, Neuss, Germany) according to the manufacturers' instructions. Plasma insulin was measured by radioimmunoassay, using rat insulin standards (Sensitive Rat Insulin Assay; Linco Research, St. Charles, MO).

**Glucose turnover studies.** After an overnight fast, animals were anesthetized (0.5 ml/kg Hypnorm [Janssen Pharmaceutica, Beerse, Belgium] and 12.5 mg/g midazolam [Gentheron, Nijmegen, the Netherlands]), and an infusion needle was placed in one of the tail veins. Then basal glucose parameters were determined, during a 30-min period, by infusion of D-[<sup>3</sup>H]glucose alone (Amersham, Little Chalfont, U.K.), after a 2-h infusion to achieve steady-state levels. In other mice, a bolus of insulin (100 mU/kg Actrapid; Novo Nordisk, Chartres, France) and D-[<sup>3</sup>-<sup>3</sup>H]glucose (60  $\mu$ Ci/kg; Amersham) was given, and a hyperinsulinemic-euglycemic clamp was started with continuous infusion of insulin (3.5 mU  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>) and D-[<sup>3</sup>-<sup>3</sup>H]glucose (0.8  $\mu$ Ci  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>). Blood samples were taken every 15 min (tail bleeding) to monitor plasma glucose levels. A variable infusion of 12.5% D-glucose (in phosphate-buffered saline [PBS]) solution was started at time 0 and adjusted to maintain blood glucose at  $\sim$ 5.0 mmol/l. During the last hour of the experiment, blood samples were taken every 20 min to determine [<sup>3</sup>H]glucose and <sup>3</sup>H<sub>2</sub>O. After the last blood sample, mice were killed and their liver, cardiac muscle, skeletal muscle, and adipose tissue were taken for analysis. To estimate the insulin-stimulated glucose transport activity in individual tissues, 2-deoxy-D-[1-<sup>14</sup>C]glucose (2-DG; Amersham) was administered as a bolus (2  $\mu$ Ci) 45 min before the end of the clamps. Blood samples were taken 5, 25, and 45 min after bolus injection to determine plasma [<sup>3</sup>H]glucose, <sup>3</sup>H<sub>2</sub>O, and 2-DG specific activities. After the last blood sample, mice were killed, and their liver, cardiac muscle, skeletal muscle, and adipose tissue were taken, directly frozen in liquid nitrogen, and kept at  $-80^{\circ}$ C for analysis.

**Tissue homogenates.** Tissue samples were homogenized ( $\sim$ 10% wet wt/vol) in PBS. To determine radioactivity of <sup>3</sup>H in the lipid fraction of cardiac and skeletal muscle and liver, lipids were extracted by a modification of Blich and Dyer's method (22). In short, 200  $\mu$ l of tissue homogenate was added to 1 ml of demi-water; then 4.5 ml of chloroform:methanol (1:2) was added and mixed thoroughly, after which 1.5 ml of demi-chloroform was added and mixed; and finally, 1.5 ml demi-water was added and mixed. After centrifugation, the chloroform layer was collected and used for determination of <sup>3</sup>H radioactivity. Total TG and cholesterol content in these homogenates was also determined in these lipids, extracted as previously described (23). The radioactivity of <sup>3</sup>H in muscle and liver glycogen was determined by digesting tissue samples in KOH and precipitating glycogen with ethanol, as described previously (24,25). For determination of tissue 2-DG uptake, tissues were homogenized ( $\sim$ 10%) in

water, boiled, and subjected to an ion-exchange column to separate 2-DG-6-phosphate (2-DG-P) from 2-DG, as previously described (26,27).

**Analytical procedures.** Plasma glucose was measured by the glucose oxidase method (Beckman Glucose Analyzer II; Beckman Instruments, Fullerton, CA). Plasma insulin was measured by radioimmunoassay, using rat insulin standards (Sensitive Rat Insulin Assay, Linco). Total plasma [<sup>3</sup>H]glucose was determined in 10  $\mu$ l plasma and in supernatants after trichloroacetic acid (20%) precipitation and water evaporation to eliminate tritiated water. The rates of glucose oxidation were determined as previously described by Koopmans et al. (28). Total plasma glucose, FFAs, TGs, and total cholesterol were determined via commercially available kits (Sigma, Boehringer Mannheim, and Wako), according to the manufacturers' instructions.

**Calculations.** Under steady-state conditions for plasma glucose concentrations, the rate of glucose disappearance equals the rate of glucose appearance. The latter was calculated as the ratio of the rate of infusion of [<sup>3</sup>-<sup>3</sup>H]glucose (dpm) and the steady-state plasma [<sup>3</sup>H]glucose specific activity (dpm/ $\mu$ mol glucose). The endogenous glucose production (EGP) was calculated as the difference between the rate of glucose disappearance and the infusion rate of D-glucose. Muscle- and adipose tissue-specific glucose uptake was calculated from tissue 2-DG-P content, which was expressed as percent of 2-DG of the dosage per gram of tissue, as previously described (25,29). Cardiac, skeletal, and hepatic muscle glycogen synthesis were calculated from <sup>3</sup>H-label incorporation into tissue glycogen, as previously described (25,29). Adipose tissue and hepatic and muscle tissue lipid synthesis from [<sup>3</sup>H]glucose was calculated from <sup>3</sup>H-label incorporation into the lipid extractable fraction, following the method of Blich and Dyer (22), as previously described (29).

**Statistical analysis.** Results are presented as means ± SD for the number of animals indicated. Differences between experimental groups were determined by the Mann-Whitney *U* test (30). The level of statistical significance of the differences was set at *P* < 0.05. Analyses were performed using SPSS 10.0 for Windows software (SPSS, Chicago).

## RESULTS

**Plasma and tissue parameters.** The body weight of LPL-overexpressing mice and control animals did not differ significantly (28.6 ± 2.7 vs. 30.6 ± 2.1 g, respectively) (Table 1). Plasma TG and cholesterol concentrations were reduced by  $\sim$ 85 and 50%, respectively (*P* < 0.05) (Table 1). FFA concentrations were found to be similar between the two genotypes in the fasted state. Fasted insulin levels were slightly increased in the LPL-overexpressing mice in comparison to wild-type mice, whereas no differences were detected in plasma glucose levels. TG content of cardiac and skeletal muscle of LPL-overexpressing mice was significantly (*P* < 0.05) increased, whereas hepatic TG content was significantly decreased (*P* < 0.05) compared with control mice (Fig. 1). There were no differences in tissue cholesterol content between the different genotypes (data not shown).

**Glucose turnover studies.** As measured by continuous [<sup>3</sup>H]glucose infusions, there were no differences in basal whole-body glucose uptake, whole-body glucose oxidation, whole-body nonoxidative glucose disposal, or EGP between LPL-overexpressing and control mice (Fig. 2A). We also performed hyperinsulinemic-euglycemic clamp studies in both mouse lines after an overnight fast. We established stable glucose levels at  $\sim$ 5 mmol/l and insulin levels at  $\sim$ 10-fold higher than under fasted conditions in

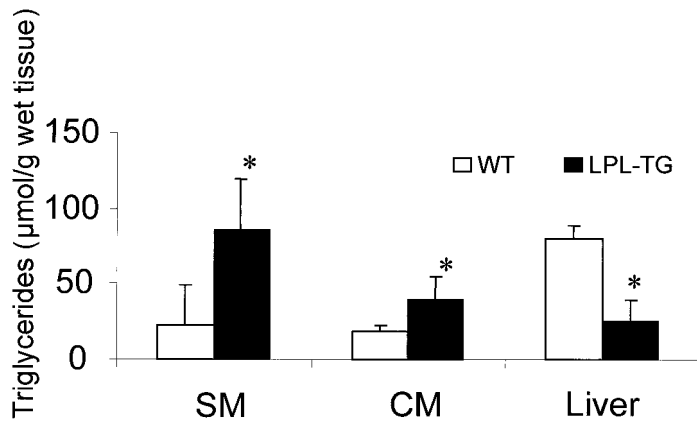


FIG. 1. Skeletal (SM) and cardiac (CM) muscle and hepatic TG (values  $\times 0.1$ ) levels determined in control (wild-type; WT) and muscle-specific LPL-overexpressing (LPL-TG) mice. Data are means  $\pm$  SD for  $n = 6$  animals per group. \* $P < 0.05$  vs. WT, as assessed by the Mann-Whitney  $U$  test.

wild-type mice, with no differences in FFA concentrations during the clamp between both genotypes (Table 2). Figure 2B shows that there were no differences in whole-body glucose uptake between both genotypes. In contrast, whole-body glucose oxidation was decreased in the LPL-overexpressing mice compared with control mice ( $P < 0.05$ ); as a consequence, whole-body nonoxidative glucose disposal was increased in LPL-overexpressing mice. The ability of insulin to suppress EGP was enhanced in LPL-

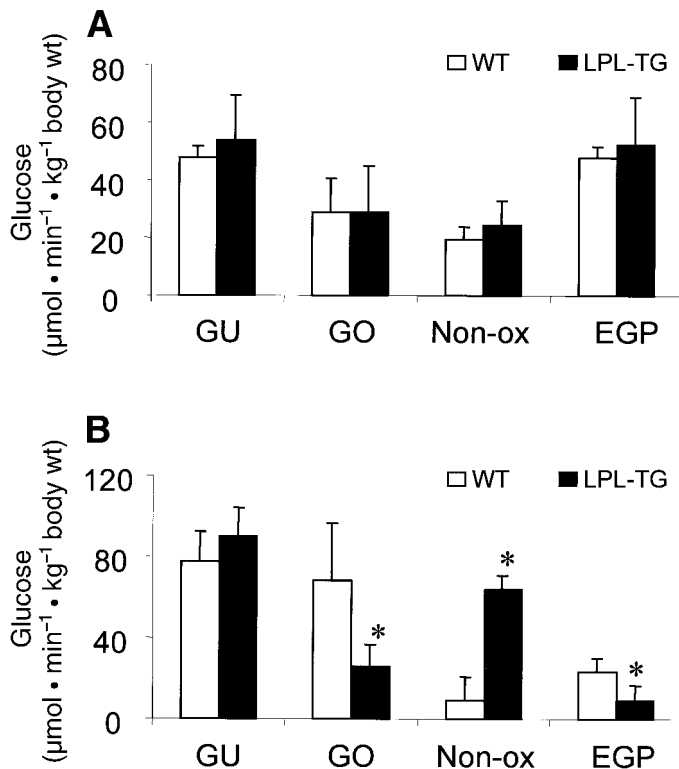


FIG. 2. Whole-body glucose uptake (GU), oxidation (GO), and storage (Non-ox) and EGP determined after overnight fasting (A) and during hyperinsulinemic-euglycemic clamp conditions (B) in control (wild-type; WT) and muscle-specific LPL-overexpressing (LPL-TG) mice. Data are means  $\pm$  SD for  $n = 6$  animals per group (A), and  $n = 10$  (wild-type) and  $n = 8$  (LPL-TG) animals per group (B). \* $P < 0.05$  vs. WT, as assessed by the Mann-Whitney  $U$  test.

TABLE 2

Concentrations of plasma glucose, insulin, and FFAs measured after hyperinsulinemic-euglycemic clamp in control (wild-type) and muscle-specific LPL-overexpressing mice

Mouse	$n$	Glucose (mmol/l)	Insulin (pmol/l)	FFA (mmol/l)
Wild-type	10	$5.2 \pm 2.1$	$433 \pm 120$	$0.60 \pm 0.19$
LPL-TG	8	$5.0 \pm 0.8$	$467 \pm 150$	$0.51 \pm 0.17$

Data are means  $\pm$  SD. No differences were found between groups. LPL-TG, LPL-overexpressing transgenic mice.

overexpressing mice compared with wild-type mice (75 vs. 40%, respectively;  $P < 0.05$ ) (Fig. 2B). Thus the liver seemed have increased insulin sensitivity because of the lower TG content (Fig. 1).

**Tissue-specific glucose uptake and glycogen synthesis.** Glucose uptake in muscle and adipose tissue was determined under hyperinsulinemic-euglycemic conditions, 45 min after a bolus of 2-DG (Fig. 3). Glucose uptake determined by 2-DG uptake was significantly increased in cardiac muscle and adipose tissue of LPL-overexpressing mice compared with controls. However, there were no differences in glucose uptake in skeletal muscle between the different genotypes. Glycogen synthesis determined by [<sup>3</sup>H]glucose incorporation into glycogen during the hyperinsulinemic clamp was not significantly different in cardiac and skeletal muscle or liver between the two genotypes. In contrast, incorporation of <sup>3</sup>H-label into the lipid extractable fraction was significantly higher in liver of LPL-overexpressing mice compared with controls ( $P < 0.05$ ), whereas skeletal and cardiac muscle and adipose tissue [<sup>3</sup>H]glucose incorporation into the lipid fraction did not differ between the two genotypes.

## DISCUSSION

Type 2 diabetes is a complex disease that is associated with strong impairment of insulin action with respect to glucose uptake—that is, insulin resistance. Increased intramuscular TG content has been found to be inversely associated with insulin action in type 2 diabetic patients (4,5,9). In lipoatrophic mice, a causal relationship between muscle TG accumulation and insulin resistance has also been found (6). The present study was performed to address the question of whether increased muscular TG

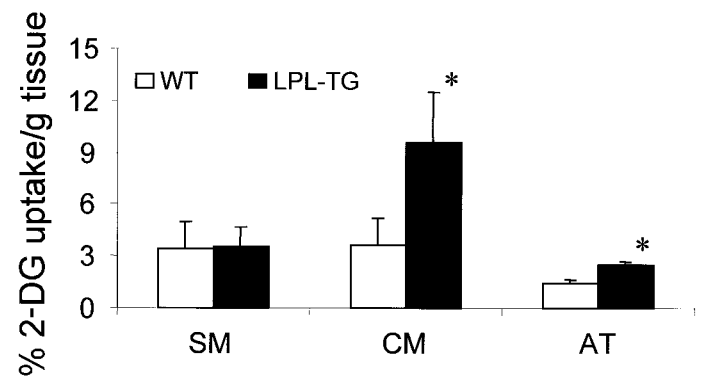


FIG. 3. Skeletal (SM) and cardiac (CM) muscle and adipose tissue (AT) glucose uptake determined with 2-DG in control (wild-type; WT) and muscle-specific LPL-overexpressing (LPL-TG) mice. Data are means  $\pm$  SD for  $n = 6$  animals per group. \* $P < 0.05$  vs. WT, as assessed by the Mann-Whitney  $U$  test.

storage is also causally related to insulin resistance in a nonobese, nonlipotrophic mouse model. To increase TG content in skeletal muscle, we used mice with muscle-specific LPL overexpression, with 2.5-fold higher total postheparin lipolytic activity and twofold higher skeletal muscle lipoprotein mass (18). Although these mice exhibited increased TG content in skeletal muscle, neither whole-body nor muscle-specific insulin-mediated glucose uptake was altered.

LPL-overexpressing mice had normal body mass characteristics compared with control mice and were not obese (16,18); however, muscle TG content was significantly higher in LPL-overexpressing mice compared with controls, a finding that agrees with data published by Weinstock et al. (16). Together with the observation that hepatic TG content was strongly decreased, these data support the role of LPL in tissue-specific partitioning of TG from TG-rich lipoproteins toward underlying tissues (20,21). Although intramuscular TG content is usually increased in the presence of obesity, high-fat feeding, and/or lipotrophy (4,6), the muscle-specific LPL-overexpressing mouse represents a useful model for examining the pathophysiological implication of increased intramuscular TG content per se without a background of obesity or lipotrophy.

Under basal, fasted conditions, there were only minor differences in plasma insulin levels between the LPL-overexpressing mice and controls, with no effects on plasma glucose levels. Furthermore, whole-body glucose uptake, oxidation and nonoxidative glucose disposal, and EGP were not different in the LPL-overexpressing mice compared with control mice. These results indicate that under fasted conditions, increased intramuscular TG content has no effect on glucose homeostasis. In addition, under hyperinsulinemic-euglycemic conditions, no differences were observed between the two genotypes on insulin-stimulated whole-body glucose uptake. These results are in line with those of Brüning et al. (31), who found no alterations in glucose uptake in muscle-specific insulin receptor knockout mice, which represent the ultimate model of extreme muscle insulin resistance.

On the other hand, under hyperinsulinemic-euglycemic conditions, EGP was found to be lower in LPL-overexpressing mice compared with controls. This observation suggests increased hepatic sensitivity with respect to the suppressive effects of insulin on hepatic glucose production. We postulate that the decrease in hepatic TG content in these mice might upregulate insulin sensitivity and thereby reduce hepatic glucose production under hyperinsulinemic conditions. Hepatic fat content correlates with impaired suppression of EGP by insulin, as described by Gupta et al. (32) and recently shown by Kim et al. (33). Liver-specific LPL overexpression leads to TG accumulation in the liver, with no obvious effects on total body insulin-stimulated glucose uptake. Furthermore, suppression of hepatic glucose production by insulin was markedly impaired in the liver LPL-overexpressing mice, as was the amount of phosphatidylinositol 3-kinase activity.

Although insulin-mediated glucose uptake was not affected in LPL-overexpressing mice, intracellular handling of glucose was altered. Whole-body glucose oxidation was strongly decreased, whereas nonoxidative glucose disposal

(glycogen and lipid synthesis) was increased in LPL-overexpressing mice compared with controls. Interestingly, these effects of muscle-specific LPL overexpression on insulin-stimulated glucose metabolism are comparable to the effects of short-term, high-fat feeding in humans (34), in whom whole-body glucose uptake was not altered, but glucose oxidation was depressed and nonoxidative glucose disposal was significantly increased.

Increased plasma FFA concentrations are inversely correlated with insulin action (35,36). Lowering of plasma FFA levels in normal and obese subjects has been shown to improve insulin action (37). In our study, no differences in plasma FFA levels were measured, neither under basal, fasted conditions nor during the hyperinsulinemic clamp study. Therefore, our study was well controlled for plasma FFA levels, and the results do not suggest impaired insulin action on lipolysis in LPL-transgenic mice.

The findings of the current study seem to contradict the inverse correlation between muscle TG content and insulin-stimulated whole-body glucose uptake found in humans (4,5,9), rats (7), and mice (6,8). However, there are distinct differences between the metabolic conditions present in our study and those of other investigators. In the human studies, the correlations were found in obese type 2 patients (4,5,9). In the rat study, the animals were treated with either a high-saturated fat diet or prolonged inhibition of fatty acid oxidation (7), and in the mouse studies, the animals were lipotrophic (6,8). Our model represents a mouse model with increased fatty acid uptake specific to muscle tissue but not subjected to long-term high-fat feeding, inhibition of fatty acid oxidation, or altered body fat content. Consequently, it must be considered that muscle TG content per se—in the absence of other alterations—does not control insulin sensitivity, in accordance with the observations in muscle-specific insulin receptor knockout mice (31).

We found that increased intramuscular TG content as a result of muscle-specific overexpression of LPL does not seem to have an effect on insulin-stimulated uptake of glucose in skeletal muscle, as determined by 2-DG uptake. On the other hand, cardiac muscle and adipose tissue glucose uptake stimulated by insulin was significantly higher in LPL-overexpressing mice compared with controls. Thus cardiac muscle and adipose tissue seem to be more sensitive to insulin with respect to glucose uptake in LPL-overexpressing mice compared with wild-type mice. An explanation for the increased insulin sensitivity of adipose tissue may be related to the small size of adipocytes found in these LPL-overexpressing mice (16), as smaller adipose cell size is related to improved insulin sensitivity (38). Additional support for our observations is the fact that muscle-specific LPL-overexpressing mice are protected from diet-induced obesity (16,39). Furthermore, increased de novo TG synthesis in these mice (16) could account for increased glucose uptake, as was found in vitro by Ruan and Pownall (40) in 3T3-L1 adipocytes overexpressing acylglycerol-3-phosphate acyltransferase (AGAT; second step in TG synthesis). Overexpression of AGAT leads to increased glucose uptake and conversion into cellular lipids in 3T3-L1 adipocytes. Because adipose tissue does not contain glycerol kinase, it depends on glucose uptake and conversion into glycerol-3-phosphate.

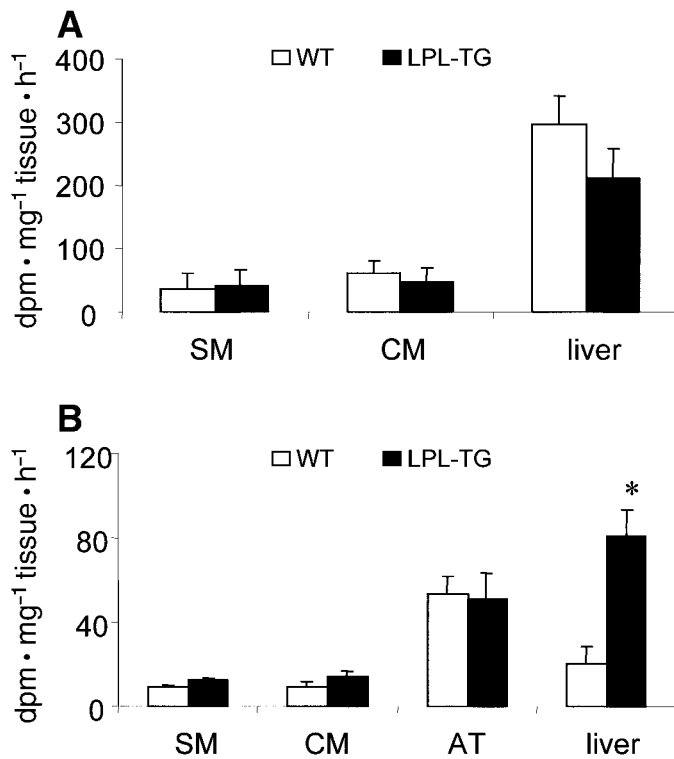


FIG. 4. Incorporation of [<sup>3</sup>H]glucose label in glycogen (A) and lipids (B) in skeletal (SM) and cardiac (CM) muscle and hepatic and adipose (AT) tissues of control (wild-type; WT) and muscle-specific LPL-overexpressing (LPL-TG) mice during hyperinsulinemic clamp conditions. Data are means  $\pm$  SD for  $n = 6$  per group. \* $P < 0.05$  vs. WT, as assessed by Mann Whitney  $U$  test.

We cannot explain the mechanism of increased cardiac glucose uptake by experimental evidence. We hypothesize, however, that in muscle-specific LPL-transgenic mice, increased amounts of fatty acids need increased glycerol for greater TG synthesis. Because cardiac muscle does not contain glycerol kinase, glycerol must be provided via glucose uptake and subsequent conversion into glycerol-3-phosphate. In vitro evidence in isolated myotubes has shown that increased TG synthesis by overexpressing AGAT increases glucose conversion to cellular lipids, with a concomitant decrease in glycogen formation (40). Our data seem to point in the same direction, although increased conversion in lipids and decreased glycogen formation (Fig. 4) were not statistically different in our study because of the relatively small numbers. Why this phenomenon is not found in skeletal muscle needs to be elucidated, but the reason may be related to the relatively low but functional glycerol kinase activity found in skeletal muscle (41).

To investigate the intracellular handling of glucose, we examined the incorporation of glucose in glycogen and lipid in the respective tissue homogenates. No significant differences were seen in skeletal and cardiac muscle and hepatic glycogen synthesis from [<sup>3</sup>H]glucose. Youn and Buchanan (24) found that decreased glucose oxidation is linked to increased glycogen synthesis, without any effect of tissue-specific glucose uptake in rats during fasting. The overnight fasting period, which induced complete depletion of the glycogen pool (24), and/or the physiological

hyperinsulinemia during the clamp analyses were sufficient to stimulate glycogen synthesis in both genotypes, showing no differences between the LPL-overexpressing and control mice. On the other hand, lipid synthesis from [<sup>3</sup>H]glucose in liver, but not in skeletal and cardiac muscle and adipose tissue, increased in LPL-overexpressing mice. The observed increased lipid synthesis from glucose in the liver may have been related to the decreased hepatic TG content in which the liver increased de novo synthesis of TGs.

The question arises how our results can be compared with recent reports by Ferreira et al. (42) and Kim et al. (33). Various obvious differences in experimental conditions can be found between their studies and ours, such as genetic background, dietary fat content, body weight, muscle and liver TG content, and insulin levels during the hyperinsulinemic-euglycemic clamp. Differences in the genetic background of the mouse model could be responsible for several metabolic and physiological differences between different mouse strains. However, it remains to be elucidated whether this explains the observed differences among the three studies. One striking difference with our study is that Kim et al. (33) did not find a decreased hepatic TG content in LPL-overexpressing mice; they did find that liver fat content is inversely correlated with the inhibitory action of insulin on hepatic glucose production (33). Ferreira et al. (42) did not describe any data on liver TG content. Therefore, additional studies are required to resolve whether differences in hepatic TG accumulation contribute to the observed differences among the three studies.

In summary, specific overexpression of LPL in skeletal muscle alters TG disposal in mice. Muscle TG content was increased in these LPL-transgenic mice, whereas liver TG content was decreased. This altered TG partitioning did not affect basal or insulin-stimulated whole-body or muscle-specific glucose uptake. Therefore, the results of this study argue against a simple causal relation between intramuscular TG accumulation and insulin resistance.

#### ACKNOWLEDGMENTS

The research described in this paper is supported by the Netherlands Organization for Scientific Research (NWO Grant 903-39-194), the Netherlands Heart Foundation (NHS Grant 97.067), and the Netherlands Diabetes Foundation.

#### REFERENCES

- Greenwood MR: The relationship of enzyme activity to feeding behavior in rats: lipoprotein lipase as the metabolic gatekeeper. *Int J Obes Relat Metab Disord* 9 (Suppl. 1):67-70, 1985
- Gruen R, Hietanen E, Greenwood MR: Increased adipose tissue lipoprotein lipase activity during the development of the genetically obese rat (fa/fa). *Metabolism* 27:1955-1966, 1978
- Kahn BB, Flier JS: Obesity and insulin resistance. *J Clin Invest* 106:473-481, 2000
- Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB, Storlien LH: Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes* 46:983-988, 1997
- Perseghin G, Scifo P, De Cobelli F, Pagliato E, Battezzati A, Arcelloni C, Vanzulli A, Testolin G, Pozza G, Del Maschio A, Luzi L: Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a 1H-13C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes* 48:1600-1606, 1999
- Kim JK, Gavrilova O, Chen Y, Reitman ML, Shulman GI: Mechanism of

- insulin resistance in A-ZIP/F-1 fatless mice. *J Biol Chem* 275:8456–8460, 2000
7. Dobbins RL, Szczepaniak LS, Bentley B, Esser V, Myhill J, McGarry JD: Prolonged inhibition of muscle carnitine palmitoyltransferase-1 promotes intramyocellular lipid accumulation and insulin resistance in rats. *Diabetes* 50:123–130, 2001
  8. Gavrilova O, Marcus-Samuels B, Graham D, Kim JK, Shulman GI, Castle AL, Vinson C, Eckhaus M, Reitman ML: Surgical implantation of adipose tissue reverses diabetes in lipotrophic mice. *J Clin Invest* 105:271–278, 2000
  9. Shulman GI: Cellular mechanisms of insulin resistance. *J Clin Invest* 106:171–176, 2000
  10. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor- $\alpha$ : direct role in obesity-linked insulin resistance. *Science* 259:87–91, 1993
  11. Hotamisligil GS, Budavari A, Murray D, Spiegelman BM: Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes: central role of tumor necrosis factor- $\alpha$ . *J Clin Invest* 94:1543–1549, 1994
  12. Olivecrona T, Liu G, Hultin M, Bengtsson-Olivecrona G: Regulation of lipoprotein lipase. *Biochem Soc Trans* 21:509–513, 1993
  13. Goldberg IJ: Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J Lipid Res* 37:693–707, 1996
  14. Farese RVJ, Yost TJ, Eckel RH: Tissue-specific regulation of lipoprotein lipase activity by insulin/glucose in normal-weight humans. *Metabolism* 40:214–216, 1991
  15. Ferraro RT, Eckel RH, Larson DE, Fontvieille AM, Rising R, Jensen DR, Ravussin E: Relationship between skeletal muscle lipoprotein lipase activity and 24-hour macronutrient oxidation. *J Clin Invest* 92:441–445, 1993
  16. Weinstock PH, Levak-Frank S, Hudgins LC, Radner H, Friedman JM, Zechner R, Breslow JL: Lipoprotein lipase controls fatty acid entry into adipose tissue, but fat mass is preserved by endogenous synthesis in mice deficient in adipose tissue lipoprotein lipase. *Proc Natl Acad Sci U S A* 94:10261–10266, 1997
  17. Eckel RH, Yost TJ, Jensen DR: Alterations in lipoprotein lipase in insulin resistance. *Int J Obes Relat Metab Disord* 19 (Suppl. 1):S16–S21, 1995
  18. Levak-Frank S, Weinstock PH, Hayek T, Verdery R, Hofmann W, Ramakrishnan R, Sattler W, Breslow JL, Zechner R: Induced mutant mice expressing lipoprotein lipase exclusively in muscle have subnormal triglycerides yet reduced high density lipoprotein cholesterol levels in plasma. *J Biol Chem* 272:17182–17190, 1997
  19. Levak-Frank S, Radner H, Walsh A, Stollberger R, Knipping G, Hoefler G, Sattler W, Weinstock PH, Breslow JL, Zechner R: Muscle-specific overexpression of lipoprotein lipase causes a severe myopathy characterized by proliferation of mitochondria and peroxisomes in transgenic mice. *J Clin Invest* 96:976–986, 1995
  20. Poirier P, Marcell T, Huey PU, Schlaepfer IR, Owens GC, Jensen DR, Eckel RH: Increased intracellular triglyceride in C(2)C(12) muscle cells transfected with human lipoprotein lipase. *Biochem Biophys Res Commun* 270:997–1001, 2000
  21. Richelsen B, Pedersen SB, Moller-Pedersen T, Schmitz O, Moller N, Borglum JD: Lipoprotein lipase density in muscle tissue influenced by fatness, fat distribution and insulin in obese females. *Eur J Clin Invest* 23:226–233, 1993
  22. Blich EG, Dyer WJ: A rapid method of total lipid extraction and purification. *Can J Biochem Biophys* 37:911–917, 1959
  23. Kuipers F, van Ree JM, Hofker MH, Wolters H, In't VG, Havinga R, Vonk RJ, Princen HM, Havekes LM: Altered lipid metabolism in apolipoprotein E-deficient mice does not affect cholesterol balance across the liver. *Hepatology* 24:241–247, 1996
  24. Youn JH, Buchanan TA: Fasting does not impair insulin-stimulated glucose uptake but alters intracellular glucose metabolism in conscious rats. *Diabetes* 42:757–763, 1993
  25. Kraegen EW, James DE, Jenkins AB, Chisholm DJ: Dose-response curves for in vivo insulin sensitivity in individual tissues in rats. *Am J Physiol* 248:E353–E362, 1985
  26. Rossetti L, Rothman DL, DeFronzo RA, Shulman GI: Effect of dietary protein on in vivo insulin action and liver glycogen repletion. *Am J Physiol* 257:E212–E219, 1989
  27. Rossetti L, Giaccari A: Relative contribution of glycogen synthesis and glycolysis to insulin-mediated glucose uptake: a dose-response euglycemic clamp study in normal and diabetic rats. *J Clin Invest* 85:1785–1792, 1990
  28. Koopmans SJ, de Boer SF, Sips HC, Radder JK, Frolich M, Krans HM: Whole body and hepatic insulin action in normal, starved, and diabetic rats. *Am J Physiol* 260:E825–E832, 1991
  29. Previs SF, Withers DJ, Ren JM, White MF, Shulman GI: Contrasting effects of IRS-1 versus IRS-2 gene disruption on carbohydrate and lipid metabolism in vivo. *J Biol Chem* 275:38990–38994, 2000
  30. Dawson-Saunders B, Trapp RG: *Basic and Clinical Biostatistics*. London, Appleton & Lange, 2001
  31. Bruning JC, Michael MD, Winnay JN, Hayashi T, Horsch D, Accili D, Goodyear LJ, Kahn CR: A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. *Mol Cell* 2:559–569, 1998
  32. Gupta G, Cases JA, She L, Ma XH, Yang XM, Hu M, Wu J, Rossetti L, Barzilai N: Ability of insulin to modulate hepatic glucose production in aging rats is impaired by fat accumulation. *Am J Physiol* 278:E985–E991, 2000
  33. Kim JK, Fillmore JJ, Chen Y, Yu C, Moore IK, Pypaert M, Lutz EP, Kako Y, Velez-Carrasco W, Goldberg IJ, Breslow JL, Shulman GI: Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. *Proc Natl Acad Sci U S A* 98:7522–7527, 2001
  34. Bisschop PHLT, de Metz J, Ackermans MT, Endert E, Pijl H, Kuipers F, Meijer AJ, Sauerwein HP, Romijn JA: Dietary fat content alters insulin-mediated glucose metabolism in healthy men. *Am J Clin Nutr* 73:554–559, 2001
  35. Perseghin G, Ghosh S, Gerow K, Shulman GI: Metabolic defects in lean nondiabetic offspring of NIDDM parents: a cross-sectional study. *Diabetes* 46:1001–1009, 1997
  36. McGarry JD, Dobbins RL: Fatty acids, lipotoxicity and insulin secretion. *Diabetologia* 42:128–138, 1999
  37. Santomauro AT, Boden G, Silva ME, Rocha DM, Santos RF, Ursich MJ, Strassmann PG, Wajchenberg BL: Overnight lowering of free fatty acids with Acipimox improves insulin resistance and glucose tolerance in obese diabetic and nondiabetic subjects. *Diabetes* 48:1836–1841, 1999
  38. Weyer C, Foley JE, Bogardus C, Tataranni PA, Pratley RE: Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts type II diabetes independent of insulin resistance. *Diabetologia* 43:1498–1506, 2000
  39. Jensen DR, Schlaepfer IR, Morin CL, Pennington DS, Marcell T, Ammon SM, Gutierrez-Hartmann A, Eckel RH: Prevention of diet-induced obesity in transgenic mice overexpressing skeletal muscle lipoprotein lipase. *Am J Physiol* 273:R683–R689, 1997
  40. Ruan H, Pownall HJ: Overexpression of 1-acyl-glycerol-3-phosphate acyltransferase- $\alpha$  enhances lipid storage in cellular models of adipose tissue and skeletal muscle. *Diabetes* 50:233–240, 2001
  41. Guo Z, Jensen MD: Blood glycerol is an important precursor for intramuscular triacylglycerol synthesis. *J Biol Chem* 274:23702–23706, 1999
  42. Ferreira LD, Pulawa LK, Jensen DR, Eckel RH: Overexpressing human lipoprotein lipase in mouse skeletal muscle is associated with insulin resistance. *Diabetes* 50:1064–1068, 2001